University of Alberta

Effects of Reproductive Timing and Paternal Care on Fathead Minnow Recruitment Dynamics

by

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Abstract

Parental care and reproductive timing can affect survival, growth, and recruitment of offspring, but may operate at different stages in the life history of fish. I examined both factors in populations of fathead minnow (*Pimephales promelas*) stocked into experimental ponds. Observed paternal care improved hatching success, especially if the male aggressively protected his nest. Although hatching success was independent of the timing of nest initiation, early-hatched fry consistently achieved larger sizes by fall than late-hatched fish, which may increase their likelihood of winter survival. Furthermore, early-hatched individuals were more likely to mature and spawn as yearlings than late-hatched fish. Thus, paternal care was important for egg survival, whereas the seasonal timing of nest activity influenced larval and juvenile life stages. To enhance understanding of fish population dynamics, assessment of factors affecting important early life stages, such as parental care and hatch dates of progeny, should be included in research protocols.

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Research is a collaborative effort, and I wish to acknowledge the people who have contributed to this project in a variety of ways over the many stages of my Master's program. I mention them chronologically to better tell the story. Being originally from Massachusetts, I thank my parents and my biology professors at Gordon College for their support and enthusiasm when I was given the opportunity to live and study in a distant (and mostly unheard of) place. My Dad and I split the driving as we journeyed across the continent to Edmonton. Upon arrival, senior lab-mate Paul Venturelli literally took me under his wing, housing me and familiarizing me with Canada, fieldwork, the ins and outs of graduate school, and life in Dr. William Tonn's lab.

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When my wife Tatyana said "yes" after I proposed to her at the Edmonton International Airport, she was unwittingly agreeing to much more than marriage. After just ten days home in New England for our August wedding, she assumed the role of an all-season field assistant and her (in)volunteering was vital on many occasions throughout the two years of the project. Tatyana's duties ranged from data recording to catching and measuring minnows. She even participated in an expedition to the remote source lake to collect fish. When times looked bleak, I needed her encouragement to press onward and her affirmation that the work I was doing was important.

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Chapter 1. GENERAL INTRODUCTION

Natural selection favors heritable traits that increase an organism's lifetime reproductive success. Evolution of behavioral traits is particularly interesting because selective pressures act on the "choices" made by organisms in like manner as physical characters. Because organisms have finite energy budgets and lifespans, many behaviors result from decisions of resource allocation; organisms seeking to maximize their inclusive fitness must decide how to invest their finite resources (Heino & Kaitala 1999). Two fundamental resource allocation decisions are when to reproduce and how much care to invest in offspring. Understanding how organisms make these choices, and being able to predict how they will respond when under different circumstances, is helpful to ecologists and resource managers with regards to population monitoring.

Reproductive timing may be governed by factors pertaining directly to the parent. First, the age (or size) at which an individual matures can be energetically constrained. In many taxa, smaller/younger individuals must postpone reproduction until they are able to meet its nutritional demands (e.g., fish: Danylchuk & Fox 1996; birds: Gonzalez-Solis et al. 2005; mammals: Langvatn et al. 2004). Where social hierarchies exist, breeding activity in subordinate individuals can also be delayed by dominant conspecifics (Cristol 1995; Danylchuk & Tonn 2001; Holand et al. 2004). Seasonality can limit the reproductive period, and can be mediated by temperature, food supply, and/or water availability (Fox 1989; Conover 1990; Rubenstein & Wikelski 2003; Loe et al. 2005).

The timing of reproduction can be further influenced indirectly through offspring performance if variation in recruitment potential exists among progeny having different birth dates. Reproductive success will be higher for parents who breed when conditions are optimal for the survival of their young. Selection on when to reproduce may be especially strong in seasonal environments, where offspring must accumulate enough energy reserves during the growing season to withstand a time when the environment becomes inhospitable (Cargnelli & Gross 1996; Ludsin & DeVries 1997; Verboven & Visser 1998). In addition, selection may favor reproductive timing that is synchronous with brief peaks in resource abundance on which offspring can capitalize (van Noordwijk et al. 1995).

Although periods of optimal breeding conditions may be somewhat predictable, stochastic variability in environmental conditions can alter these dates among years. Some of these temporally variable factors, e.g., food or habitat availability, or predator abundance (Donovan et al. 1997; Dzus & Clark 1998), may even fluctuate independently of seasonal climatic patterns. Consequently, many species overcome this environmental uncertainty by adopting a "bet-hedging" reproductive strategy (Amundsen & Slagsvold 1998; Garvey et al. 2002). By producing multiple clutches intermittently across an extended reproductive period, the probability of having at least some progeny recruit is increased, thereby improving fitness.

Parental care, defined as any post-fertilization investment of time and energy into progeny (Gross & Sargent 1985), is a second behavior that influences reproductive success. Parental duties vary widely across taxa and include incubation, nest defense, and feeding of hatchlings. According to life history theory, care for offspring should only evolve if there is a net increase in fitness of the caregiver (Gross & Sargent 1985). If mortality of offspring is inherently high, the benefits of ensuring that the present young

survive may outweigh the costs of providing care, namely energetic expenditure and foregone production of additional progeny (Gross & Sargent 1985; Paez et al. 2004; Salomon et al. 2005).

In most fishes that provide parental care, parents clean, aerate, and guard their eggs from predators until hatching (Blumer 1979). Paternal care (i.e., performed by the father alone), the most common type of parental care in oviparous fish, is believed to have evolved from female nest site selection (Gross & Sargent 1985). Because freshwater fishes typically spawn demersal eggs, females can choose where to oviposit (Gross & Sargent 1985). Males that control the best spawning substrates receive the most spawnings and, as holders of territories that are attractive to females, they suffer no mating costs in providing nest care (Gross & Shine 1981; Gross & Sargent 1985). Guarding males tend the nest and protect it from predators, which include female conspecifics in several species (FitzGerald & Whoriskey 1992; Vandenbos 1996; Hishida 2002), likely reinforcing this mating system. Interestingly, paternal care facilitates fractional spawning; since females are released from a commitment to care for their eggs, they are able to concentrate their efforts on feeding and can therefore produce additional clutches (Blumer 1979).

I examined the consequences of both reproductive timing and parental care on offspring performance in the fathead minnow (*Pimephales promelas*), a protracted spawner that exhibits solitary male care. In a two-year study, I introduced minnows into experimental ponds at Meanook Biological Research Station (MBRS; Alberta, Canada) on two stocking dates. In Chapter Two, I report effects of hatch date on early life stages by tracking survival and growth of age-0 cohorts hatching in each stocking treatment through their second summer of life. In Chapter Three, I focus on the hatching success of nests produced by these experimental populations. By intensively monitoring nesting activity, I was able to compare egg survival among nests receiving different amounts of paternal care.

Addressing these questions at the mesocosm level had several advantages compared to other spatial scales. Egg production and hatching success are difficult to quantify at the population-level in aquatic systems. On the other hand, laboratory studies, using aquaria or larger tanks, fail to incorporate complex ecological interactions that naturally influence spawning dynamics and recruitment. By using small experimental ponds, I could obtain both precise estimations of egg survival while preserving a realistic spawning scenario that incorporated abiotic and biotic phenomena, such as temperature regime, demographic changes, and community interactions, naturally experienced by populations. Insight into the importance of reproductive timing and parental care on survival in the early life stages of progeny should enhance our understanding of population dynamics in fish.

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Chapter 2. IMPORTANCE OF REPRODUCTIVE TIMING AND HATCH DATE FOR FATHEAD MINNOW RECRUITMENT

Introduction

Variation in the timing of reproduction can affect population dynamics in fish (e.g., Ludsin & DeVries 1997; Garvey et al. 2002) and has a variety of natural causes. Interannual variability in environmental conditions, e.g., winter duration, can alter dates of spawning initiation by regulating the time of year water reaches the minimum temperature at which spawning can occur (Mills 1991; Danylchuk & Tonn, in press). Differences in energetic condition among spawners can also affect reproductive timing. After ice-off, larger/older offspring may be in better energetic condition and able to invest more towards reproduction. In contrast, smaller/younger individuals may require more time to replenish a relatively larger energy deficit and are unable to spawn until later in the summer (Goodgame & Miranda 1993; Danylchuk & Fox 1994, 1996). Third, inhibition of spawning can also occur through social control, whereby smaller, subordinate males may not initially breed because they are prevented from establishing nesting territories by dominant conspecifics (Danylchuk & Tonn 2001). Lastly, for fractional, or multiple-batch, spawners, fish breed repeatedly throughout the season, allowing even the same parents to produce offspring that differ in hatch date.

The benefits of hatching early result from the ability of young-of-the-year (YOY, or age-0) fish to attain a larger body size due to a longer growing season (Keast & Eadie 1984). Large age-0 fish are less vulnerable to gape-limited predation than small individuals (Pine et al. 2000). Additionally, because they have wider gapes, larger young have a wider variety of prey types available to them (Phillips et al. 1995; Ludsin & DeVries 1997). Third, large YOY escape the allometries associated with smaller sizes that are detrimental to first-winter survival. These include low capacity for energy storage (primarily in the form of lipids), disproportionately higher mass-specific metabolic requirements (Bernard & Fox 1997; Cargnelli & Gross 1997; McCollum et al. 2003), and a larger gill-surface to body ratio susceptible to osmoregulatory failure in frigid waters (Fullerton et al. 2000; McCollum et al. 2003). Size-biased predatory or starvation mortality, selecting against smaller size-classes, can often result from these handicaps (e.g., Post & Evans 1989; Garvey et al. 1998; Schultz et al. 1998; Braaten & Guy 2004). Therefore, YOY produced from earlier spawnings can have higher first-year survival than those hatching later in the summer (Keast & Eadie 1984; Cargnelli & Gross 1996; Ludsin & DeVries 1997; Conover et al. 2003). Direct evidence of improved performance of early-hatched age-0 fish has been largely limited to the first summer of life or to assessment of first-winter survival, although a few longer-term studies have shown that earlier-hatched YOY can mature at younger ages than later-hatched conspecifics (Baylis et al. 1993; Saito & Nakano 1999). Hatch-date related differences in growth, survival, and age of maturity may ultimately result in differential fitness among offspring within a cohort.

However, optimal timing of reproduction can shift due to variability in environmental conditions that may unpredictably alternate between favoring early or late hatch dates. For example, in years when spring conditions are characterized by cool water temperatures, low food availability, or high abundance of predators, survival rates of early-hatched YOY may be lower than offspring produced later in the season (Pine &

Allen 2001; Garvey et al. 2002; Santucci & Wahl 2003). The temporally variable sources of mortality facing offspring may make it adaptive for parents to spread out egg production over time, ultimately selecting for phenotypic plasticity in breeding schedules (Schultz 1993; Garvey et al. 2002).

Since cold water temperatures, extended winter duration, and starvation can be major causes of first-winter mortality in fish (Post & Evans 1989; Fullerton et al. 2000), recruitment in northern fish populations may be especially sensitive to delayed spawning periods. The objectives of my research were to examine how the temporal dimension of spawning influences recruitment dynamics in boreal fishes. In a two-year study using experimental ponds, effects of reproductive timing on performance of progeny were compared between treatments of relatively early- and late-spawning fathead minnow populations. I hypothesized that over an extended spawning season, earlier-hatched YOY would grow larger and be in superior condition at ice-on than later-hatched counterparts, which would result in higher first-winter survival. By following cohorts for two years, I was also able to examine longer-term effects of hatch date on year-class strength. I predicted that growth advantages associated with earlier hatch dates of age-0 minnows would be maintained through their second summer as age-1 fish.

Study Organism

The broad range of the fathead minnow (Cyprinidae: *Pimephales promelas*) extends throughout most of North America. In the boreal lakes of Alberta, which represent the northern geographical limit (Nelson & Paetz 1992), single-species assemblages of fathead minnow are not uncommon (Price et al. 1991; Danylchuk & Tonn 2003), and fish predominately occupy shallow ($\leq$2 m depth) littoral habitats (Danylchuk & Tonn, unpub. data).

Both fathead minnow's regional abundance and breeding habits facilitate in-depth population studies. Fathead minnow are promiscuous batch-spawners, producing several clutches of eggs over the summer months (Gale & Buynak 1982; Scott & Crossman 1998). Females deposit adhesive eggs on the underside of aquatic vegetation or woody debris that are fertilized and then guarded by the males (McMillan & Smith 1974; Nelson & Paetz 1992). Fathead minnows will also readily spawn on artificial substrates (e.g., Benoit & Carlson 1977; Grant & Tonn 2002; Clemment & Stone 2004), enabling detailed observation of nest activity and hatching success that are not easily obtained from other species.

Danylchuk & Tonn (2003, in press) described a stable, boreal Alberta population of fathead minnow inhabiting South Calling Lake 100 (SCL100; 55° 5′ N, 113° 47′ W), a small waterbody devoid of piscivorous fish. Both male and female minnows matured at age-3 and lived up to age-5 (Danylchuk & Tonn, in press). Dates of spawning initiation ranged from 22 May - 18 June, when water temperatures reached at least 17°C, and average duration of spawning activity was 69 d (Danylchuk & Tonn, in press). I collected fish from the SCL100 population for my experiment.

Methods

Study Site.—In 2003 and 2004, I used four experimental ponds (Ponds 3-6; surface area = 178 ±18.1 m^2, $\bar{X} \pm$ SE; 1.5 m maximum depth), located at Meanook Biological Research Station (MBRS; 54° 37′ N, 113° 35′ W), near Athabasca, Alberta, Canada. To control for

possible differences among ponds that might confound the results, I divided the ponds in half with water-impermeable polyethylene tarpaulin supported by wooden posts (sensu Grant & Tonn 2002). Each pond-half was then designated as either Early-Stock (ES) or Late-Stock (LS), and I alternated treatment groups in ponds used in both study years. To equalize hydrostatic pressures that were problematic in the first summer, I inserted four small rectangular sections (total area ~1.5 m^2) of fine-mesh (0.56 mm) screens into the tarpaulin curtains of all ponds used in 2004.

Natural spawning substrate, i.e., aquatic vegetation and coarse woody debris, was removed from the ponds before stocking. I then anchored 11 floating fenceboards (1.6 x 0.14 m) along the shoreline in each pond half (total spawning surface available = 2.47 m^2). These "nestboards" were covered in black tarpaulin to aid egg detection. A submersible temperature logger (Onset Corp., Pocasset, MA, USA) recorded water temperature hourly 0.3-0.5 m below the surface of each pond.

Experimental Procedures.—I collected fathead minnows in spring from SCL100 using funnel traps. Fish were transported to MBRS, where they were kept in shaded, outdoor holding tanks and fed maintenance rations of flake-food. Length-frequency distributions (measured to the nearest 1 mm total length, TL) were determined separately for three life history categories: Male, Female, and Immature (defined as fish lacking distinguishable secondary sexual characteristics; see Scott & Crossman 1998). Fathead minnows categorized as Immature may mature and reproduce before the end of the current spawning season (Grant & Tonn 2002; W.M. Tonn & C.A. Paszkowski, unpub. data). Based on mean TL of fish for each category (n = 364-1132), I subdivided the categories into Small, Medium, and Large size-classes (3-mm intervals) and then batch-marked fish with fin-clips or subcutaneous injection of acrylic paint. Minnows were segregated into the holding tanks by sex to suppress spawning activity.

I separated initiation of the spawning season of experimental populations of fathead minnows by staggering their stocking dates into the ponds by three weeks. On 24 June 2003, even ratios of life history categories and size-classes of fathead minnows were introduced into one ES pond-half in each of three experimental ponds (Ponds 3, 4, and 5) at a natural density of approximately 1 fish/m^2 (23.3 kg fish/ha; Danylchuk & Tonn 2003), based on the average surface area of the ponds. The three LS pond-halves were stocked with the same population structure on 15 July. Minnows found dead within 12 d of stocking (just as spawning commenced) were replaced by fish of the same category and size from the holding tanks. I removed surviving stocked minnows in late August by extensive trapping. Details of and comparisons between ES and LS populations are presented in Appendix A.

I inspected nestboards for eggs daily over the duration of the spawning season and recorded histories of all nests in detail. Egg masses were enumerated with the aid of a counting grid, and all eggs were ultimately accounted for as either diseased, depredated, or hatched (sensu Vandenbos 1996; Grant & Tonn 2002).

Biweekly from July to Oct 2003, I sampled "Early-Hatch" (EH) and "Late-Hatch" (LH) age-0 fish by performing standardized seine sweeps (seine: 1 x 1.2 m, 2 mm mesh) and setting three minnow traps overnight in each pond-half. Age-0 minnows were counted and measured (TL) to calculate first-summer growth and relative abundance. The 2003 year-class then remained in the ponds over the winter. I monitored pond dissolved oxygen (DO) levels periodically under the ice throughout the winter. Pond-

halves became progressively hypoxic as winter progressed, so I aerated the ponds on three occasions in February to increase dissolved oxygen (DO) stores.

From 13-20 May 2004, I drained the ponds down to depths of 0.3-0.5 m and pond-widths of ~4 m. I then removed all age-0 fish by seining repeatedly (seine: 4.5 x 1.2 m, 2 mm mesh) until three consecutive hauls yielded zero fish. All YOY were counted and measured, and subsamples were weighed to assess overwinter survival and condition. Ponds were refilled and the experiment was repeated in Ponds 5 and 6, with stocking of ES and LS treatments again staggered by three weeks (17 June and 8 July 2004, respectively).

In late Aug 2004, I harvested YOY from all pond-halves by the drain-and-seine technique described above. All fish were counted to estimate cohort abundance, and subsamples were measured (TL) and weighed (nearest mg). Fulton's condition factor (K = 100 x [mass(g) / length(cm)^3]) was then calculated as a measure of general health.

In May 2004, following the post-winter removal of age-0 fish, I reserved Pond 4 for comparing EH and LH performance of the 2003 year-class through their second year of life. After draining Pond 4, overwinter survivors (now age-1 fish) were seined out of the pond and temporarily held in holding tanks, until being re-stocked at equal densities (160 fish/pond-half) on 7 June 2004. None of these fish exhibited signs of sexual maturation at time of re-stocking. I added eight nestboards to each pond-half (0.48 x 0.09 m; total spawning surface available = 0.34 m^2), and monitored spawning activity and egg production as above. On 26 July, age-1 fish were trapped to assess growth (TL and wet mass), condition (K), and were examined for development of secondary sex characteristics. Sub-samples of mature females were sacrificed to measure their gonadosomatic index (GSI = ovarian mass / total mass x 100). The pond was drained on 11 Aug, and all age-1 fish, along with their offspring, were removed by seining for final comparisons between EH and LH treatments.

Statistical Analyses.—Using two-factor, Model III (mixed-model) ANOVAs, I tested for treatment effects (the fixed factor, ES vs. LS, or EH vs. LH) on several reproductive and growth-related measures. Pond was the random factor when comparisons were made across multiple ponds; sampling date was the random factor when Pond 4 fish were analyzed over multiple dates. Because 2003 YOY were measured in multiple ponds over multiple dates, ANOVAs were performed separately for each sampling date. Natural logarithms of the probabilities obtained from these individual tests were then summed to generate a G-like statistic, which was compared to a χ^2 distribution to detect overall treatment effects (Sokal & Rohlf 1995). Because the use of only two ponds for the experiment in 2004 reduced the df of the denominator MS to one, pond-wise t-tests were substituted for ANOVA for comparing growth parameters of age-0 fish between treatments. For these tests, unequal variances were corrected by the Welch-Satterthwaite approximation of degrees of freedom (Zar 1999).

To identify the degree to which growth and size-selective first-winter mortality influenced cohort size structure, I examined quantile-quantile (QQ) plots of fall and spring YOY TL distributions (sensu Post & Evans 1989). I used G-tests of independence to compare survival rates of age-0 fatheads, and percent maturity of age-1 fish between treatments. If necessary, length, count, and proportion data were, respectively, log, square root, and arcsine-square root transformed to meet the conditions of normality required for parametric testing. When such assumptions could not be met, I used

Wilcoxon signed-rank (T), Mann-Whitney (U), or Kruskal-Wallis (H) tests as nonparametric alternatives. Significance levels for all analyses were set at $\alpha = 0.05$. When multiple comparisons were made among groups, significance levels of the pairwise tests (α') were adjusted, using the Dunn-Sidak method, to maintain this experiment-wise error rate (Sokal & Rohlf 1995).

Results

Reproduction.—At time of stocking, water temperature was higher when LS fatheads were added to the pond-halves than for ES fish, by 7°C and 2°C in 2003 and 2004, respectively. In 2003, daily water temperature averaged over the duration of the ES spawning and nesting seasons was 20.6°C. However, mean water temperature in 2003 LS pond-halves was 22.2°C, almost 2°C warmer than all other treatment-years. In 2004, temperatures of ES and LS pond-halves were similar, averaging 20.4°C.

ES spawning commenced approximately 9 d after stocking in both years of study. LS reproduction began 6 d post-stocking in 2003, and 12 d post-stocking in 2004 (Table 2-1). Once initiated, spawning occurred daily over the next ca. 24 d for ES minnows, about 6 d longer than the LS breeding season (Table 2-1). Consequently, LS egg production extended 2-3 weeks later into the summer than in the ES populations. Mean nest size was similar between treatments (Table 2-1). Although not statistically significant (signed-rank tests: p's > 0.14), total ES egg production and nest-days exceeded those of LS in four of five pond-years. Average number of ES and LS nests was identical in 2003, but the difference in egg production was more pronounced in 2004 because of relatively few LS nests (Table 2-1). In general, eggs hatched within 6-8 d of deposition in both treatments. Hatching success, the proportion of eggs that hatched per nest, was marginally higher in LS pond-halves for both years (Table 2-1).

Age-0 Fish.—Despite slight discrepancies in LS spawning initiation between 2003 and 2004, overlap in hatch dates of EH and LH age-0 fathead minnows within each pond was minimal in both years (Table 2-1). Differences in peak hatch-out between EH and LH YOY, defined as the period of four consecutive days with the highest number of Stage 3 eggs (just prior to hatching; Vandenbos 1996), ranged from 11-26 d for all pond-years. The maximum difference in age within EH and LH age-0 cohorts was 27 d, as determined by the duration of hatching.

By fall 2003, EH YOY were 13-89% greater in length than LH fish in all three pond-pairs, with these differences being significant on all sampling dates (t-tests: t = 3.38-11.29, p's ≤ 0.001; n = 29-89; Fig. 2-1). According to the ANOVA, fish size differed significantly between treatments for two of these sampling dates (12 Aug to 18 Oct 2003) where more than 25 individuals were captured in each pond-half (Fig. 2-1). Moreover, the significance of the summed probabilities of all four ANOVA tests (G_T = 21.40, df = 8, $p = 0.006$) revealed a clear overall effect of hatch date on YOY length.

In late Aug 2004, LH YOY were just over half the length of their EH counterparts in Pond 5 (Table 2-2). However, growth differences in YOY from Pond 6 were not as distinct: LH YOY averaged only 1.2 mm (7%) smaller than EH offspring (Table 2-2). The variation in YOY sizes between treatments for Ponds 5 and 6 created a highly significant pond-treatment interaction term ($p < 0.0001$) that masked any detectable differences in length in the ANOVA. However, according to pond-wise t-tests, LH age-0

fish had significantly smaller lengths than EH fish in both Ponds 5 and 6 (Table 2-2). Mass and condition of LH YOY were significantly lower (87% and 14%, respectively) than that of EH fish in Pond 5 (Table 2-2). However, mean condition of Pond 6 LH YOY was actually 7% higher than in EH counterparts (Table 2-2).

Mass of 2003 age-0 fatheads sampled on 13 Sep could be estimated using a length-mass log-linear regression of age-0 fish measured late-Aug 2004 (log[wet mass(g)] = 3.237 x log[TL(mm)] - 5.423; $r^2 = 0.96$, $n = 391$). Fish measured on 13 Sep 2003 were chosen because the 2004 year-class would have been most similar in age to the 2003 year-class on that date, due to later stocking in 2003 than in 2004. As was observed for length, predicted mass and condition of EH YOY were also greater than in LH 2003 cohorts

Upon final draining of the two ponds studied in fall 2004, YOY abundances varied greatly among pond-halves. In Pond 6, the catch of EH YOY was three times greater than in the LH side, while Pond 5 had 25% fewer EH individuals than the LH age-0 cohort (Table 2-2). Despite this variation, total EH age-0 biomass was approximately five times greater than that of the LH cohorts in both ponds (per unit of pond surface area; Table 2-2). First-summer survival was significantly higher for LH age-0 minnows than for EH YOY in both ponds, and averaged 33.5% and 19.5% for LH and EH treatments, respectively (*G*-tests of independence: p's < 0.0001; Table 2-2).

The relationship between number of hatchlings and fall cohort size observed in the four 2004 pond-halves was determined by linear regression. Although low sample size meant that the positive relationship was not significant ($r^2 = 0.68$, df = 2, $p = 0.18$), I used the regression equation to estimate fall abundance of age-0 minnows in each pond-half in 2003. While differences in estimated EH and LH cohort size varied among these ponds, expected biomass density of EH age-0 fish was 2-4 times higher than that of LH cohorts.

Overwinter.—Ice cover lasted 177 d (26 Oct 2003 - 20 Apr 2004) on the MBRS ponds. By 15 Feb, increasing ice thickness decreased water depth to 0.25-0.5 m, and water temperatures fell below 2°C. Likewise, DO concentrations declined to 0.2-0.5 mg/L, but aeration efforts successfully increased these levels, at least temporarily, up to 3-4 mg/L. Still, winter mortality of age-0 fish was severe, with overall losses of 64%, 78%, and 100%, for Ponds 3, 4, and 5, respectively, based on estimated fall 2003 abundances. Total spring harvests of Pond 3 YOY was 2,604 fish, with all but ~30 individuals caught in the EH pond-half. Unfortunately, Pond 3's divider had breached under the weight of the heavy snowfall and overflowed from snowmelt, so contamination between treatments through mixing of fish was suspected. Thus, I used only Pond 4 overwinter survivors for the 2004 age-1 experiment.

In Pond 4, twice as many EH age-0 minnows survived winter than did LH offspring (793 EH vs. 390 fish, respectively). EH YOY had slightly higher overwinter survival than LH fish (24.3% vs. 18.3%, respectively), as calculated from estimated cohort abundances in fall 2003. However, total first-year survival, based on 2003 hatching success, was similar between treatments (~5%) because fewer LH hatchlings were produced. Despite their higher spring density, EH survivors were 16% (3.4 mm) longer and 66% (50 mg) heavier than LH individuals. Mean TL (± SE) of EH fall (18 Oct 2003) and spring (20 May 2004) minnows were 18.7 ± 0.5 and 24.6 ± 0.3 mm, while

LH fish were 16.6 ± 0.5 and 21.2 ± 0.2 mm, respectively (Fig. 2-2a,c). Based on QQ plots, both EH and LH groups demonstrated some growth between these dates, presumably during the 30 d period after ice-off before fish were measured. However, the shallow slope of the LH plot indicated a disproportionate loss of smaller individuals from that cohort (Fig. 2-2d) relative to the EH fish (Fig. 2-2b).

Age-1 Fish.—In 2004, both EH and LH cohorts (now age-1 fish) grew ~33 mm, more than doubling their spring size (Fig. 2-3) and increasing in mass 15-fold. 4-5 mm length differences between treatments persisted into their second summer (nine sampling periods; $F_{1,8} = 24.62$, $p = 0.001$; Fig. 2-3). Additionally, for the May, July, and August sampling dates in 2004, EH immature age-1 fish (lacking secondary sex characteristics) were significantly heavier and in better condition than LH age-1 fish (mass: $F_{1,2} = 30.44$, $p = 0.031$; condition: $F_{1,2} = 267.77$, $p = 0.004$). By 11 Aug, mass and condition of immature age-1 fish were 31% and 5% greater, respectively, in EH fatheads than in LH fish.

Overall, mature individuals, primarily males, had greater length, mass, and condition than their immature counterparts (Kruskal-Wallis: p's < 0.0001), but such differences were independent of treatment (U-tests: p's > 0.1; Fig. 2-4). However, significantly more EH age-1 fish exhibited signs of maturity than LH minnows on both July and August sampling dates (G-tests of independence: p's ≤ 0.0005; Table 2-3a). GSI values for mature EH females were also 3.4 times greater than values for mature LH females in late July (U-test: $p = 0.017$; Table 2-3a). Both EH and LH age-1 cohorts demonstrated high second-summer survival (Table 2-3a), relative to end-of-season survival of the parental populations (21%, App. A). Age-1 females were 16% (10 mm) smaller and 44% (1.23 g) lighter than Females stocked in Ponds 5 and 6 (U-tests: p's $<$ 0.0001; App. A,B).

Spawning commenced for both age-1 cohorts on 15 July. EH spawning duration (16 d) extended 10 d longer than the LH season, and nest and egg production were also higher in EH than in the LH treatment (Table 2-3b). Although EH eggs started hatching 4 d later and extended 12 d beyond that of LH, EH offspring were 33% longer and almost 2.5 times heavier than LH progeny at final removal on 11 Aug (Table 2-3c). Age-0 abundance was slightly higher and, based on the predicted hatch, survivorship was also greater in the LH pond-half (G-test of independence: $p < 0.0001$; Table 2-3c). However, given the larger sizes of EH offspring, biomass density of YOY was 2.6 times greater in the EH than the LH pond-half (Table 2-3c).

One LS Large Female that had been added to Pond 4 during the 2003 experiment apparently escaped both fall removal trapping and spring seining, and was not removed until after the LH age-1 spawning season (on 26 July). She appeared to be gravid and was twice as heavy as the LH age-1 females.

Discussion

Reproduction.—Dates of ES spawning initiation in the MBRS ponds were 1-2 weeks later than those documented in SCL100 fathead minnow (Danylchuk & Tonn, in press). This delay can be attributed to the interruptions to the minnows' natural spawning patterns by a combination of transportation, tank-induced, and handling stresses, followed by later re-stocking in June. Cooler pond water temperatures in July and

August of 2004, possibly a result of greater rainfall compared to 2003 over that same period (Climate Data Archive, Environment Canada), may partially explain the reduction in LS fecundity in 2004 relative to that of 2003 LS fatheads.

Because ES and LS hatching overlapped only slightly, the ages of the majority of offspring differed by about two weeks between treatments, while maximum age differences approached 30-40 d. Such seasonal variation in hatch dates is not only feasible for this species, but can be even more extreme in natural populations, where spawning seasons can extend longer than two months (Nelson & Paetz 1992; Danylchuk & Tonn, in press). The discrepancy in spawning season may be understood by considering the greater abundance of the SCL100 fathead minnow population ($\bar{X}$ = 62,490 individuals; Danylchuk 2003) compared to that of the MBRS ponds (84-95 fish/pond-half). I expect that SCL100 had a correspondingly greater number of reproductively active fish contributing to overall egg production than the experimental populations, which may spawn over a longer period of time.

Age-0 Fish.—In accord with my hypothesis, delayed spawning in LS fathead minnows influenced the next generation of fish, decreasing first-summer size of LH progeny, likely because later hatch dates limited their seasonal growth potential (Keast & Eadie 1984; Cargnelli & Gross 1996). However, the additional influence of density-dependent factors on growth cannot be ruled out. High cohort density may have partially stunted the growth of EH YOY in Pond 6, explaining why these progeny were in poorer condition than the LH group. Despite considerable variation in cohort densities, EH YOY were consistently longer and comprised a greater biomass than LH counterparts in all five pond-years.

Survival of age-0 minnows was not related to numerical density, but was higher in both LH cohorts by fall (2004), and therefore appeared inversely related to fish biomass. Alternatively, higher fall survival in LH treatments may have been because LH age-0 fish were approximately three weeks younger than EH fish at time of removal. In this view, EH progeny had a higher probability of dying simply because of longer exposure to risk factors (Garvey et al. 2002).

Pond 3, containing the largest age-0 fish, had the lowest overall winter mortality (64%), while no age-0 fatheads survived winter in Pond 5, likely due to a combination of starvation and anoxia. Pond 4 suffered 78% mortality, and size-selective starvation appears to at least partially explain the positive size shift in fatheads, especially for LH fish. Minimum fall sizes of YOY were 11 mm, but no fish less than 16 mm survived their first winter. The LH cohort had a greater proportion of individuals under this minimum-size threshold (52.3% LH vs. 38.6% EH, respectively). Therefore, starvation mortality may have been more threatening to smaller LH YOY than for their older, larger EH counterparts. This would also explain the shallower slope of the LH QQ plot relative to that of the EH plot. Growth occurring prior to the spring stock assessment (30 d after ice-off) may have also shifted the age-0 size structure, apparent from the position of the QQ distributions above the reference lines (Post & Evans 1989).

Age-1 Fish.— Effects of hatch-date on fathead minnow performance carried over into the second year of life, as predicted, with EH winter survivors remaining consistently larger than LH fish; no compensatory growth in LH minnows was observed. This size

advantage may account for why proportionally more EH age-1 fish matured and also had greater gonadal investment and fecundity than LH fish. Maturation at age-1 was unexpected because the minimum age of maturity documented for SCL100 fathead minnow was age-3 (Danylchuk & Tonn, in press). However, when Pond 4 age-1 minnows were measured on 26 July, fish had achieved sizes ($\bar{X}$ = 50 mm TL) equivalent to age-3 minnows from SCL100 (Danylchuk & Tonn, in press).

Maturation of Pond 4 fish at ages two years younger than that observed in their lake of origin indicates that age-at-maturity can be size-dependent in fathead minnow. Indeed, the age at which fish mature is often phenotypically plastic and decreases with growth rate (Stearns & Koella 1986; Fox 1994; Heino et al. 2002). A single cohort of age-0 minnows was introduced to Pond 4 at a relatively low density, enabling accelerated growth rates which, in turn, facilitated maturation as yearlings. Interestingly, the life history pattern of the Pond 4 cohort resembled that of fathead minnows occupying two other lakes situated within 5 km of SCL100, where maturation also occurred at age-1 (Danylchuk & Tonn, in press). In these two lakes, average densities of fathead minnow were 7-38% that of SCL100 (Danylchuk & Tonn 2003). Consequently, average lengths of age-1 fish were significantly (6-10 mm) greater in these two other lakes than for SCL100 fish (Danylchuk & Tonn in press).

Life history contrasts among fathead minnow populations implicate the role of density-dependence in the timing of maturation. Low stock densities, such as occurring following a winterkill, can decrease the age of maturity by accelerating juvenile growth (Fox & Keast 1991; Danylchuk & Tonn, in press). Conversely, transition to adulthood may be delayed when higher population densities result in resource limitation and/or competitive interactions that retard juvenile growth (Fox 1994). Furthermore, higher densities of older males may socially repress age-1 males from breeding in SCL100 (Danylchuk & Tonn 2001).

It is possible that the carryover adult Female from 2003 deposited some eggs on the single LH nest. If LH age-1 reproductive effort was, in fact, overestimated because of her egg contribution, then true differences between age-1 treatments were even more extreme than what was observed.

Reproductive effort of the age-1 cohort, as measured by spawning duration and fecundity, was markedly less than the effort of populations of older LS adult Females in Ponds 5 and 6 that began spawning on similar dates. This difference in reproductive investment was probably size-linked; age-1 females in Pond 4 were significantly smaller than the Females stocked in other ponds. Another indication of limited reproductive effort was the fact that end-of-summer survival of Pond 4 age-1 cohorts was over three times higher than that of the adult populations stocked in other ponds, with catch rates of mature age-1 fish remaining stable over time. These results may suggest that mature age-1 fatheads had negligible post-spawning mortality after their first breeding season because of reduced reproductive investment.

Performance of EH versus LH cohorts beyond age-1 was not investigated in this study, and it is possible that maturation at age-1 may have consequences for future growth and reproduction (Heino & Kaitala 1999). Though reproductive effort observed in age-1 fathead minnows was small relative to larger fish, energetic costs associated with spawning and paternal care could potentially decrease the lifespan of EH age-1 fish relative to LH individuals that delayed maturation, as has been shown in pumpkinseed

sunfish (Fox 1994). Long-term studies are needed to examine the effects of seasonal timing of spawning on the entire life history of offspring.

The offspring of the age-1 fish (Pond 4) were comparable to LH age-0 fish from other ponds (born of older fathead minnows), which had similar hatch dates. Surprisingly, however, reproductive timing of adult fish in 2003 may have also affected the second generation of fathead minnow. Progeny of EH age-1 fish were significantly longer than offspring of LH minnows, even though hatching of the former began several days after the latter. However, because the EH offspring hatched out over a longer time interval and had lower survivorship, they may have experienced reduced intra-cohort competition. In any case, replication would be needed to validate these findings.

Implications of Reproductive Timing

Fluctuations in both environmental and demographic conditions can alter the timing of spawning which, in turn, can affect performance of the new year-class. Fish spawning early in the season may demonstrate higher fitness if their offspring are able to outperform YOY hatched later in the season. By having greater opportunity for growth before the onset of winter (Cargnelli & Gross 1996), early-hatched YOY are larger, making them less vulnerable to predatory invertebrates and gape-limited piscivorous fish, including cannibalism by older conspecifics (Vandenbos 1996; Garvey et al. 1998; Pine et al. 2000). Another benefit of earlier hatch dates is the capability of older age-0 fish to switch to larger prey earlier than younger, smaller fry (Phillips et al. 1995). This can ensure food availability for early-hatched YOY by expanding the options of prey items accessible to them (Ludsin & DeVries 1997). With a greater assemblage of high-quality food available to larger fish, early emergence may be especially important for first-year recruitment where winter duration and severity impose a survival bottleneck on fish populations (Ludsin & DeVries 1997). In northern climates, small YOY could starve after their limited energy reserves become depleted during long, cold winters (Schultz et al. 1998; Fullerton et al. 2000). Smaller body size of late-hatched YOY may result in particularly low overwinter survival, especially where size-dependent winter mortality operates. Finally, early spawning may be advantageous, from a fitness standpoint, if early-hatched age-0 fish are able to mature and spawn at a younger age because of their superior size and condition to later-hatched fish.

However, potential advantages of early hatch dates may be undermined by stochastic environmental perturbations or predation, which may act to decrease survival rates of early-hatched age-0 fish. Seasonal fluctuations in water levels can increase mortality of early-hatched age-0 fish by altering the size and quality of shoreline habitat that can be utilized by these offspring (Kohler et al. 1993). Cool spring temperatures and limited prey abundance can also slow growth rates of early-hatching offspring relative to those produced later in the season, though complete growth compensation in late-hatched fish is rare (Ludsin & DeVries 1997; Pine & Allen 2001; Garvey et al. 2002). Where piscivores are present, early-hatched fry can also suffer high predatory mortality because they are the first prey available at a time when prey fish are scarce (Donovan et al. 1997; Pine & Allen 2001). Additionally, early-spawned YOY are exposed to predators for a longer period than later hatches and therefore have greater probability of being eaten (Santucci & Wahl 2003).

Conclusions

Trade-offs surrounding the decision of when to reproduce are contingent on changing environmental factors. To combat temporally variable threats on age-0 survival, fractional spawning has been adopted by cyprinids and other fishes as a type of bet-hedging strategy (Garvey et al. 2002). Spawning intermittently across the growing season can improve fitness in variable environments by maximizing the likelihood of at least some offspring recruiting. Producing multiple clutches also increases total fecundity, especially in small-bodied fishes where reduced coelomic space limits egg production (Mills 1991).

Reproductive timing should be considered from a geographic perspective. Arbitrary designation of "early" and "late" hatches are latitudinally relative, dependent on local climate and length of the growing season. Even across the broad geographical distribution of fathead minnow, dates of spawning initiation range from March to mid-June for southern and northern populations, respectively (Andrews & Flickinger 1974; Scott & Crossman 1998). In other freshwater species, the entire spawning season can end by June at lower latitudes (e.g., Phillips et al. 1995; Pine & Allen 2001), where late-hatched age-0 fish experience long growing seasons and mild winters. In contrast, the spawning season may not even begin until late May or June at higher latitudes, and the growing season is markedly shorter, and long, harsh winters pose a greater threat to YOY (e.g., Garvey et al. 2002; Santucci & Wahl 2003; Danylchuk & Tonn, in press). Therefore, recruitment in boreal fish populations may be especially sensitive to the seasonal timing of reproduction.

Reproductive timing is the initial stage that regulates recruitment in fish (Ludsin & DeVries 1997) and can be factored into assessments of population dynamics in the following ways. First, it would be beneficial for fisheries biologists and managers to identify lake-scale disturbances likely to delay spawning of all members of the population. Events such as extended ice-up may indicate "late-hatch years" that could weaken recruitment of age-0 fish. Second, researchers working with species that are protracted spawners should attempt to monitor nesting activity and identify seasonal transitions among spawning individuals in the population. Smaller or younger fish that spawn late in season would contribute little to the population if few of their young recruit (Danylchuk & Fox 1994). Third, greater emphasis needs to be placed on identifying sub-cohorts (i.e., classifying fish by the month of emergence) in addition to year-classes. Assessing individuals at this finer time scale would be especially important if advantages bestowed on early-hatched YOY persist to maturation and improve their fitness relative to late-hatched progeny. Knowledge of spawning conditions, seasonal reproductive patterns, and relative abundances of early and late age-0 sub-cohorts will enhance predictive power in forecasting strong versus weak year-classes.

Table 2-1. 2003 and 2004 spawning season dates and characteristics of Early-Stock (ES) and Late-Stock (LS) fathead minnow populations inhabiting MBRS ponds, averaged across ponds. Values are given as $\bar{X} \pm$ SE. Nest-days are the summation of the number of nests present each day over the entire reproductive seasons. Nest size and hatching success are followed by results of Model III ANOVAs comparing ES and LS treatments for each year. In 2003, spawning and hatching in a single LS nest in Pond 5 continued until 18 Aug and 22 Aug, respectively.

	2003 (Ponds 3, 4 & 5)		2004 (Ponds 5 & 6)	
	ES	**LS**	**ES**	**LS**
Pond stock date (Julian d)	24 Jun (175)	15 Jul (196)	17 Jun (169)	8 Jul (190)
Spawning season (Julian d)	3 Jul - 25 Jul (184 - 206)	21 Jul - 6 Aug (202 - 218)	25 Jun - 20 Jul (177 - 202)	20 Jul - 7 Aug (202 - 220)
Spawning duration (d)	23 ± 2	17 ± 1	26 ± 2	19 ± 1
# of nests	15 ± 4	15 ± 2	25 ± 2	6 ± 0
Nest-days	139 ± 26	118 ± 18	160 ± 17	67 ± 7
Nest size (# eggs)	2,337 ± 247	1,915 ± 226	1,292 ± 114	1,740 ± 391
	$F_{1,2} = 1.06, p = 0.41$		$F_{1,1} = 0.18, p = 0.75$	
Seasonal egg prod. (# eggs)	34,270 ± 7,716	28,093 ± 6,599	32,304 ± 4,738	10,439 ± 3,684
Hatching season (Julian d)	10 Jul - 27 Jul (191 - 208)	27 Jul - 8 Aug (208 - 220)	30 Jun - 24 Jul (182 - 206)	26 Jul - 14 Aug (208 - 227)
# hatchlings produced	14,224 ± 743	15,360 ± 4,703	17,108 ± 4,116	7,257 ± 3,454
Hatching success (%)	30.6 ± 4.6	41.5 ± 5.0	39.9 ± 5.6	62.0 ± 9.6
	$F_{1,2} = 13.83, p = 0.065$		$F_{1,1} = 64.60, p = 0.079$	

Table 2-2. Cohort and individual fish characteristics for Early-Hatch (EH) and Late-Hatch (LH) age-0 fathead minnows in MBRS ponds, at the end (25 Aug) of the 2004 season. Values for length, mass, and condition are given as the $\bar{X} \pm$ SE. Treatments with higher means, determined from pond-wise *t*-tests, are denoted as follows: * $p < 0.05$, *** $p \leq 0.0005$.

Pond	Stock	Abundance	Survival (%)	# fish/m^2	g fish/m^2	TL (mm)	Mass (mg)	Condition (*K*)	*n* (TL , mass)
5	EH	2,732	21.0	26.3	3.65	24.5 ± 0.2***	170 ± 8***	0.79 ± 0.01***	723 , 116
5	LH	3,656	34.1	40.7	0.70	12.6 ± 0.1	22 ± 2	0.68 ± 0.01	904 , 96
6	EH	3,814	18.0	46.2	2.47	18.1 ± 0.1***	75 ± 5***	0.79 ± 0.02	843 , 96
6	LH	1,252	32.9	12.1	0.51	16.9 ± 0.2	48 ± 3	0.85 ± 0.02*	144 , 83

Table 2-3. Comparisons between Pond 4 Early-Hatch (EH) and Late-Hatch (LH) (a) age-1 fish (b) their seasonal reproduction, and (c) their offspring during 2004. Age-1 minnows were sampled in July by standardized trapping, while the entire age-0 and age-1 cohorts were captured in August by repeated seining. (c) Individual-level characteristics of age-0 fish, measured in August, are given as $\bar{X} \pm$ SE. Treatments with higher means, determined from pond-wise *t*-tests, are denoted as follows: * $p < 0.05$, *** $p < 0.001$.

A.	Date	Stock	# fish	Survival (%)	# fish/m^2	g fish/m^2	# mature	% mature	♀ GSI (%)
age-1	26 Jul	EH	81	-	-	-	7♂, 9♀	19.8	9.83, 7
		LH	118	-	-	-	1♂, 4♀	4.2	2.90, 3
	11 Aug	EH	94	58.8	1.2	2.36	11♂, 14♀	26.6	-
		LH	118	73.8	1.6	2.34	1♂, 1♀	1.7	-

B.	Stock	Spawning season	Spawning duration (d)	# of nests	Nest size ($\bar{X}$ # eggs)	Seasonal egg prod. (# eggs)	Hatching success (#, %)
reproduction	EH	15 Jul - 30 Jul	16	3	1,315	3,946	2,197, 42.3
	LH	15 Jul - 20 Jul	6	1	1,689	1,689	830, 49.1

C.	Stock	# fish	Survival (%)	# fish/m^2	g fish/m^2	TL (mm)	Mass (mg)	Condition (*K*)	*n* (TL, mass)
age-0	EH	363	16.5	4.7	0.62	25.0 ± 0.3***	109 ± 8***	0.68 ± 0.02*	225, 78
	LH	416	50.1	5.7	0.24	18.8 ± 0.1	44 ± 2	0.62 ± 0.01	260, 78

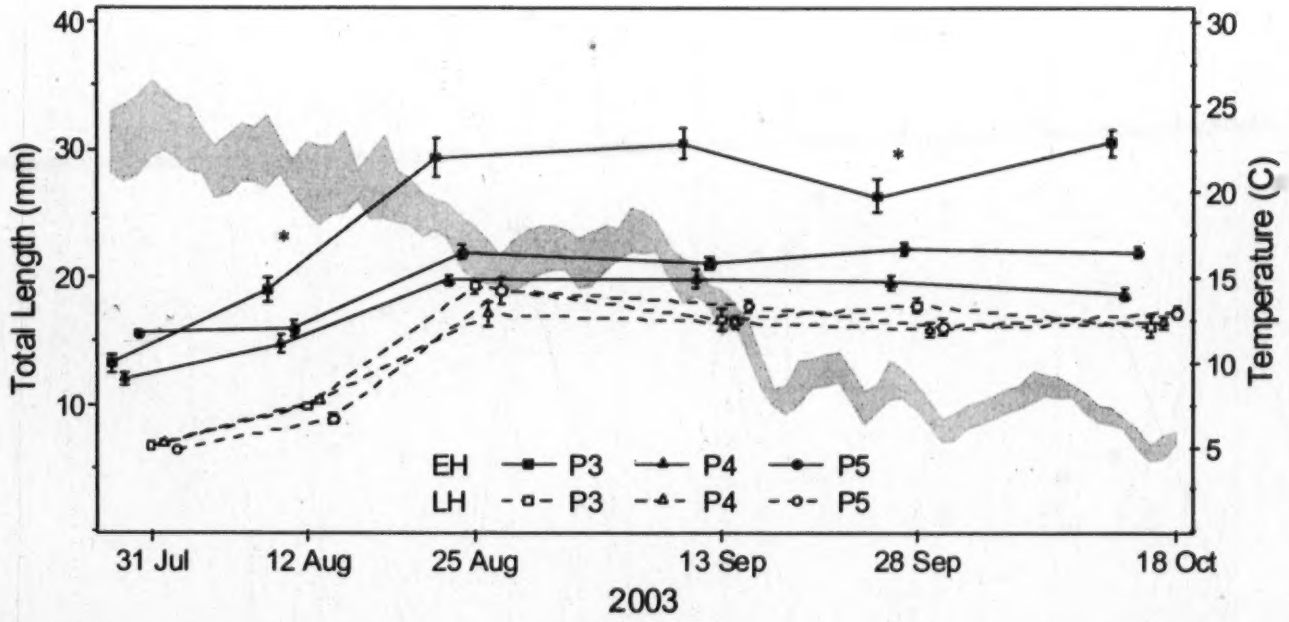

Figure 2-1. First-summer growth of Early-Hatch (EH; solid shapes and lines) and Late-Hatch (LH; open shapes/dashed lines) age-0 fathead minnows for the three ponds in 2003. Data are presented as $\bar{X} \pm$ SE. The shaded region encompasses the daily range in water temperature. Growth apparently ceased by mid-September when mean temperatures fell to 12°C. EH age-0 fish were consistently larger than LH offspring in all ponds over the six sampling periods. Model III ANOVAs were conducted on 12 Aug, 13 Sep, 28 Sep, and 18 Oct. Asterisks designate sampling dates where treatment effects were significant ($p < 0.05$). ANOVA was not performed on 31 July or 25 Aug because of few fish captured in some of the LH pond-halves.

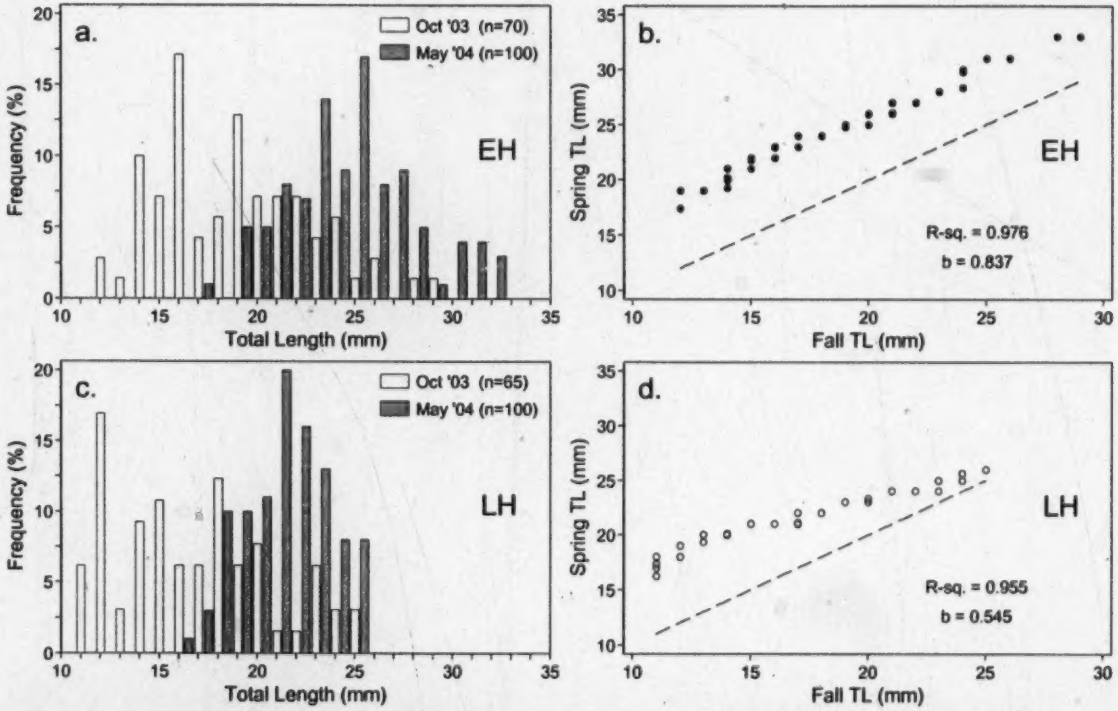

Figure 2-2. Length-frequency histograms (left panels) with respective quantile-quantile (QQ) plots (right panels) comparing fall and spring total length (TL) distributions of Pond 4 YOY: (a & b) Early-Hatch (EH) and (c & d) Late-Hatch (LH) cohorts. The QQ plots include coefficients of determination (r^2) and slopes (b) from linear regression. Dashed lines are 1:1 references representing no change in size structure overwinter. Values above these lines indicate growth; slopes <1 suggest a loss of smaller individuals.

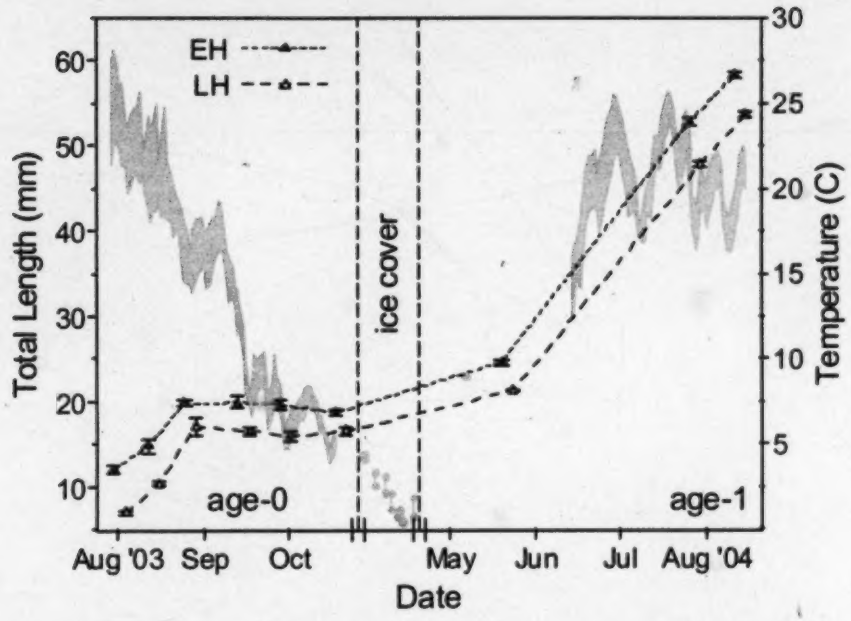

Figure 2-3. Growth of Early-Hatch (EH; solid triangles) and Late-Hatch (LH; open triangles) fathead minnows in Pond 4 across two summers. Data are presented as $\bar{X} \pm$ SE. The shaded region (or dots in winter) encompasses the daily range in water temperature. Two scale breaks along the x-axis delimit a compressed time scale during winter.

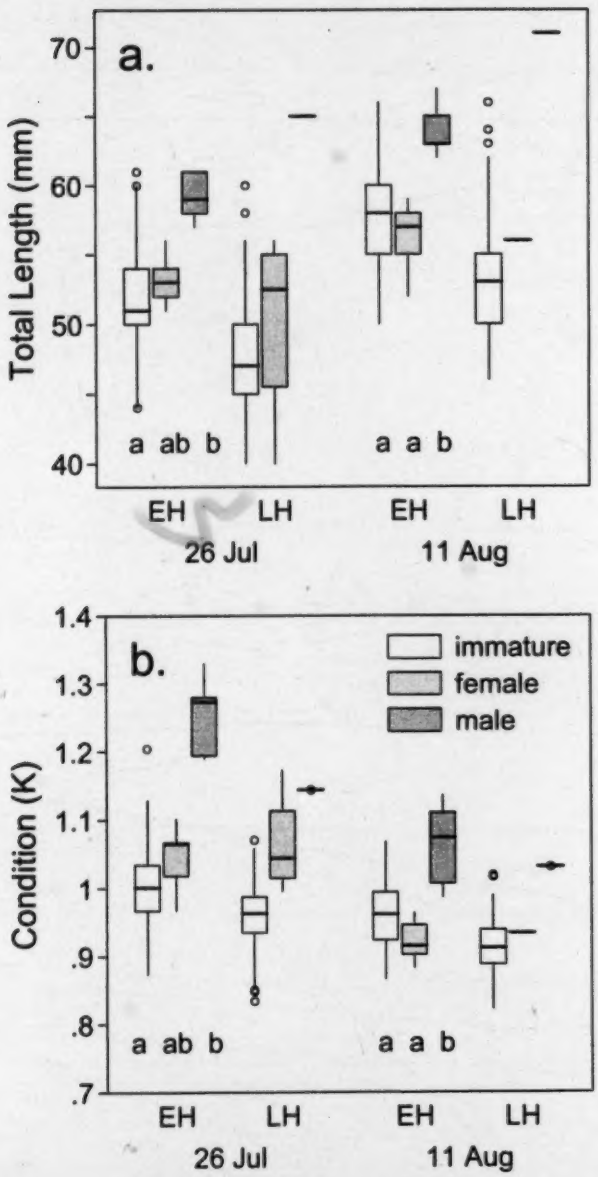

Figure 2-4. (a) Total length (TL) and (b) condition (*K*) of age-1 fathead minnows from Early-Hatch (EH) and Late-Hatch (LH) cohorts in July and August 2004. Different letters denote EH groups that were significantly different ($\alpha' = 0.017$) using Dunn's multiple comparison tests after Kruskal-Wallis (Zar 1999). Because of the scarcity of mature LH individuals, analyses were restricted to the EH cohort. Sample sizes are presented in Table 2-3a.

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Chapter 3. EFFECTS OF SEASONAL REPRODUCTIVE TIMING AND PATERNAL CARE ON HATCHING SUCCESS IN FATHEAD MINNOW

Introduction

Understanding the high degree of variability in year-class strength, commonly seen in fish populations, remains a central issue in fisheries research. Several stages early in life have been identified as barriers to recruitment because fish are extremely vulnerable to mortality during their first year (Houde 1987; Houde 1989; Santucci & Wahl 2003). For example, the transition to exogenous feeding and the first-winter period are two interdependent episodes when offspring are highly susceptible to starvation and/or predation (Donovan et al. 1997; Ludsin & DeVries 1997; Garvey et al. 2002; McCollum et al. 2003). Mortality rates of age-0 fish entering these critical periods may dictate recruitment potential of the cohort and subsequently affect overall population dynamics (Houde 1987; Pine et al. 2000).

However, due to the high fecundity of most fishes (Kamler 1992; Wootton 1992), rates of mortality at the pre-larval stage, i.e., the period of embryonic development as eggs, may also be an important factor determining the strength of new year-classes. Unfortunately, this egg stage is often overlooked in natural systems because of the difficulties in monitoring egg production and survival at the population level. Hatching success, defined as the proportion or number of eggs that complete development and hatch, is highly variable in many fish populations and can lead to dramatic interannual fluctuations in year-class strength (Bradford & Cabana 1997). Because of fitness benefits associated with successful recruitment of progeny, natural selection should favor behaviors of reproductively active adults that optimize egg survival. Two major ways that breeding individuals influence hatching success are when they spawn and to what degree they invest in caring for their eggs.

Seasonal timing of reproduction may affect hatching success with regard to changing abiotic and biotic conditions. Length of egg incubation is generally inversely related to water temperature (Mills 1991), and can therefore vary depending on when eggs were spawned. Longer incubation may lengthen the period that eggs are vulnerable to mortality factors such as disease or predation. High densities of fish or invertebrates capable of eating eggs can also reduce egg survival (Vandenbos 1996). Seasonal changes in the abundance of egg predators may thus alter the hatching success of eggs laid at different times during the spawning season. Finally, seasonal fluctuations in water levels may also influence egg survival. Eggs spawned before a flooding event could suffocate from siltation or, alternatively, may dessicate from exposure if the water level recedes (Kohler et al. 1993).

Parental care can be generally defined as any post-fertilization investment of time and energy by a parent into its offspring (Gross & Sargent 1985). Parents that provide care can improve their reproductive success by increasing the survival rates of their progeny. Brood care is common in fish and except in cases where fertilization is internal, the father is predominately the caregiver (Blumer 1979; Gross & Sargent 1985). Types of care performed by breeding males can vary across taxa (see Blumer 1979), but all activities share two major functions: 1) protecting the brood from predation, and 2) cleaning the eggs to improve hatching success. Although paternal care is believed to

have initially evolved from male territoriality over prime spawning sites (Gross & Shine 1981), selection for this behavior is reinforced in species where females are egg cannibals. Adult females can devour entire clutches if nests are unguarded (Unger 1983; FitzGerald & Whoriskey 1992), making paternal care critical for egg survival in these species.

The main objectives of this study were to determine how seasonal timing of reproduction and paternal care affected hatching success in fathead minnow. I also examined whether differences in the degree of nest defense and care performed by males further influenced hatching success. Secondary objectives were to characterize attributes of nest site selection, temporal shifts in spawning activity among breeding males, and sources of egg mortality. I predicted that nests produced later in the summer would have higher egg survival because of warmer water temperatures as well as a lower abundance of egg predators, primarily due to the post-spawning mortality of conspecifics (Flickinger 1973). I also hypothesized that hatching success would be positively related to paternal care. Understanding interrelationships between timing of nest activity and nest care should provide insight into determinants of hatching success, as well as help predict how variation in these behaviors may subsequently affect year-class strength.

Study Organism

The breeding habits of fathead minnow (*Pimephales promelas*), a cyprinid common throughout much of North America, permit researchers to acquire detailed histories of spawning activity and hatching success that are not easily obtained from other fishes. Fathead minnows typically spawn adhesive eggs on the undersides of aquatic vegetation or woody debris (Nelson & Paetz 1992). Fatheads will also utilize a variety of artificial substrates for egg deposition (e.g., Jensen et al. 2001; Grant & Tonn 2002; Clemment & Stone 2004), making the nests readily accessible for study.

In northern populations, spawning commences in late May or early June and extends until August (McMillan & Smith 1974; Nelson & Paetz 1992; Danylchuk & Tonn, in press). At the beginning of the spawning season, mature males become territorial about an object suitable for egg deposition and, after spawning, care for eggs until they hatch (McMillan & Smith 1974; Nelson & Paetz 1992). Throughout the incubation period, the male cleans and aerates the eggs by frequently rubbing them with a spongy pad protruding from the pre-dorsal region of his body (McMillan & Smith 1974). Several rows of keratinized bumps, called nuptial tubercles, encircle the mouth of the male and are used to defend the nest against a host of egg predators, including female conspecifics, as well as to ward off rival males from usurping his territory (Unger 1983; Smith 1991; Vandenbos 1996; Scott & Crossman 1998).

Fathead minnows are fractional, or multiple-batch, spawners, with females typically producing new clutches of eggs every 3-4 d (Gale & Buynak 1982; Jensen et al. 2001), which greatly increases their overall fecundity across the breeding season. For fathead minnows held in outdoor tanks, Gale and Buynak (1982) reported average clutch sizes of 400-500 eggs, varying widely from nine to over 1,000 eggs/clutch, with more than 10,000 eggs laid by some females over an entire summer. Fatheads are promiscuous; nests of individual males often consist of multiple clutches that were spawned by several females, while females can deposit eggs in several nests (Scott & Crossman 1998). Post-spawning mortality in breeding adults is often high (Flickinger

1973; Unger & Sargent 1988), presumably a result of high energetic expenditure from parental care in males and high reproductive investment in females. As the spawning season progresses, breeding individuals that have died are replaced by smaller/younger minnows that have recently matured (Grant & Tonn 2002). Fractional spawning and turnover of reproductively active individuals lead to extended spawning, nesting, and hatching seasons.

Methods

Pond Stocking.—In 2003 and 2004, I used four small (~180 m^2) experimental ponds (2003: Ponds 3, 4, and 5; 2004: Ponds 5 and 6), located at Meanook Biological Research Station (MBRS; 54° 37′ N, 113° 35′ W), near Athabasca, Alberta, Canada. I divided ponds in half by installing water-impermeable tarpaulin curtains (sensu Grant & Tonn 2002), and designated each pond-half as either Early-Stock (ES) or Late-Stock (LS). I removed aquatic vegetation and coarse woody debris from the ponds before stocking to restrict potential spawning substrate in each pond half to 11 floating fenceboards (1.6 x 0.14 m) that I anchored along the shoreline (total spawning surface available = 2.47 m^2). To enhance egg visibility, these "nestboards" were covered in black tarpaulin.

Fathead minnows used in this experiment were acquired from SCL100 (55° 5′ N, 113° 47′ W; surface area 18 ha; mean depth 3.5 m; see Danylchuk & Tonn 2003). Collected fish were grouped into Male, Female, and Immature categories, and Male minnows were divided into the following 3-mm size-classes: Small, 65-67 mm total length (TL); Medium, 69-71 mm; and Large, 73-75 mm. In 2004, Males of each size-class were given unique paint-marks adjacent to the origin of the dorsal fin for size identification. Equal proportions of fathead minnows from all categories were added to the ponds to produce an average population density of 1 fish/m^2. Fish were stocked into ES and LS pond-halves in June or July on dates that differed by three weeks (Fig. 3-1). Temperature loggers (Onset Corp., Pocasset, MA, USA) were deployed ~0.4 m below the surface of each pond and recorded water temperature hourly.

Nest Monitoring Protocol.—After stocking, I inspected the nestboards daily for eggs, and the duration of the spawning/nesting seasons were delimited by the presence of eggs on this substrate. I mapped relative locations of the egg masses on the boards, and recorded histories of all nests in detail. Eggs were enumerated with the aid of a counting grid (1 cm^2 divisions), printed onto a transparent acetate sheet. Where egg cover was 100%, 60 eggs filled a single grid square. To count egg masses, I turned over a nestboard, keeping the eggs submerged, and clipped the grid in place over the nest. Positive differences in day-to-day egg counts over the duration of a nest's existence determined the total nest size; negative differences indicated net losses to hatching or predation. Egg mortality from disease was also quantified by counting the number of eggs that became white and covered with fungal hyphae. Nest duration was defined as the number of days that eggs were present in a nest site. Mean nest temperatures were calculated from daily temperature readings taken over the duration of each nest. In 2004, 2-3 eggs from each nest were collected on dates that were within the first two weeks of the ES and LS spawning seasons (2 July and 31 July, respectively), fixed in Bouin's solution, and later measured at 30x magnification to the nearest 0.03 mm.

Hatching success, or egg survival, was defined as the proportion of fertilized eggs in a nest that successfully developed and emerged from the egg envelope as free-

swimming embryos (see Penaz 2001). To estimate this parameter, I tracked egg developmental stages according to a 3-stage classification system (sensu Vandenbos 1996; Grant & Tonn 2002). Bright, gold-colored irises indicated that embryos reached the third and final stage of development and would hatch within 24-48 h, depending on the water temperature. Because Stage-3 eggs often remain on the nestboard for 1-2 d before hatching, hatching success was calculated by quantifying specific regions of a nest where Stage-2 eggs were first observed reaching Stage-3. A nest was considered to have "failed" if none of its eggs survived.

Measuring Paternal Care.—During the daily nest surveys, nests were considered cared for if they were seen being cleaned and defended by male minnows either before I overturned nestboards or while I counted eggs. In addition to the presence/absence of a male guardian, the extent of guarding was calculated as a proportion of time by dividing the number of days males were present by the nest's longevity. Males were labeled as Aggressive if they displayed agonistic behaviors, e.g., charging, butting, or biting (see McMillan & Smith 1974), when their territory was intruded during nestboard inspections. Based on these observations, all nests were ultimately classified as having one of the following three paternal care categories: No Care, Passive Care, or Aggressive Care. In 2004, size-classes of the territorial males were also recorded, if discernable.

Statistical Analyses.—I performed linear regressions to test for relationships between hatching success and the following independent variables: water temperature, the timing of nest activity, nest size, nest duration, and proportion of care that nests received. Nests were pooled among ponds to increase sample size, and regressions were analyzed separately for each of the four treatment-years: 2003 ES, 2003 LS, 2004 ES, and 2004 LS.

Influence of paternal care on three dependent variables - nest size, nest duration, and hatching success - was analyzed using three-factor, Model III (mixed-model) ANOVAs, setting year (2003 and 2004) as the random factor; level of paternal care (No Care, Passive Care, and Aggressive Care) and stocking treatment (ES and LS) were fixed factors. Differences among group means were tested for significance by the Tukey-Kramer method of pairwise comparisons for groups with unequal sample sizes (Zar 1999). For a more general analysis of care, Passive and Aggressive Care nest categories were pooled and compared against No Care using *t*-tests, corrected for unequal variance using the Welch-Satterthwaite approximation of degrees of freedom (Zar 1999). To meet the assumptions of parametric statistics, count and proportion data were square root and arcsine-square root transformed, respectively.

I tested for directional nest site preference using replicated goodness-of-fit *G*-tests (Sokal & Rohlf 1995), comparing proportions of nests located on either northern or southern shorelines in each pond-half. Nest failure was compared among levels of paternal care with a *G*-test of independence, adjusted for continuity with William's correction (Sokal & Rohlf 1995). Differences in aggressiveness among male size-classes were also analyzed using this test.

I compared reproductive timing and egg survival among size-classes of male caregivers using nonparametric Kruskal-Wallis tests, followed by Dunn's multiple-comparison tests for groups having unequal sample sizes (Zar 1999). Significance levels for all analyses were set at $\alpha = 0.05$, and for multiple comparisons, Dunn-Sidak

adjustments were made to comparison-wise significance levels (α') to protect against Type-I error (Sokal & Rohlf 1995).

Results

Nest Activity.—In all, 150 nests were produced over both years of study. Nesting was asynchronous across nesting seasons that spanned ca. 20-30 d (Table 3-1). Average nest size was 1,817 ± 114 (SE) eggs, but ranged from four to over 7,300 eggs. Eggs hatched within 6-8 d of deposition (Table 3-1), but because successive clutches were laid in many nests, 18% of all nests lasted 13 d or more, and duration of four nests exceeded 20 d. At the (pond-half) population level, eggs were laid continuously throughout the spawning seasons, although I consistently observed pulses of egg production, occurring at ca. 3 d intervals (Fig. 3-1). It was common to see eggs of various developmental stages within a single nest, and the appearance of new eggs, coupled with the disappearance of older eggs (either because of mortality or hatching), led to daily variation in nest sizes (Fig. 3-2).

Daily water temperatures averaged over the duration of ES and LS spawning and nesting seasons were similar in 2004 and, consequently, so were average incubation periods (two-factor model III ANOVA: $F_{1,2} = 2.42$, $p = 0.36$; Table 3-1). However, mean water temperature in 2003 LS pond-halves was almost 2°C warmer than all other treatment-years, resulting in incubation periods that were 1.5 d less for LS eggs than for ES eggs in that year ($F_{1,2} = 17.96$, $p = 0.051$; Table 3-1). Diameter of eggs laid in LS nests was 6.2% larger than that of ES eggs in 2004 ($t = 5.34$, $p < 0.0001$, $n = 23$-26; Table 3-1).

Eggs exhibiting signs of fungal infection were found in 37% of all nests. However, in nests where fungus was present, only 2% of the eggs, on average, were infected. The average proportion of egg mortality due to fungus was 3.5% across all treatment-years.

The proportion of nests that failed, i.e., had 0% hatching success, was 29% overall, and slightly greater in nests produced in ES versus LS populations (sign-rank test: $p = 0.043$, $n = 5$ pond-years; Table 3-1). Hatching success was highly variable with an overall average of 39.4 ± 2.9% ($\bar{X}$ ± SE). There was little biological significance detected in the relationship between hatching success and either mean nest temperature ($r^2 \leq 0.05$, df = 10-48, p's ≥ 0.13) or date of nest initiation ($r^2 \leq 0.22$, df = 10-48, p's ≥ 0.002; Fig. 3-3), although statistical significance was reached in some cases due to large sample sizes. In contrast, hatching success was (marginally) positively related to both nest size and nest duration in three of four treatment-years (2004 LS excluded; nest size: $r^2 = 0.33$-0.36, df = 42-48, p's < 0.0001; nest duration: $r^2 = 0.40$-0.60, df = 42-48, p's < 0.0001; Fig. 3-4). Most nests that failed disappeared shortly after their initiation, but the duration of 25% (11/44) of the failed nests met or exceeded the minimum incubation period observed (5 d, $n = 11$ nests).

Nest Site Selection.—During the peak of the ES and LS spawning seasons, no more than 73% of nestboards in each pond-half contained egg masses. Breeding males selected territories in close proximity to each other; 69% of the nests were simultaneously active with at least one other nest on individual nestboards, with 18% sharing a nestboard with

two other nests. The average number of nests on active nestboards exceeded one in each pond-year (1-sample *t*-tests: $t = 4.42\text{-}9.03$, 1-tailed p's < 0.0001; $n = 26\text{-}34$).

No nest site preference was detected between nestboards situated on northern versus southern shorelines; 47 and 44 nests were observed on northern and southern nestboards, respectively, in eight pond half-years ($G_T = 6.47$, df = 8, $p = 0.59$; Pond 4 was excluded because of an unbalanced placement of nestboards on the north and south shorelines).

Paternal Care.—I observed paternal care, occurring for at least a portion of a nest's existence, in 81% of all nests. These male guardians often quickly returned to their nests and resumed caretaking duties, i.e., scrubbing and circling the eggs, after my nestboard inspections. Of all territorial males observed, 35% displayed agonistic behaviors. Aggressive males charged, butted, and nibbled the clip that held the counting grid, as well as fingers and/or legs of recorders.

Level of paternal care, i.e., No Care, Passive Care, or Aggressive Care, influenced nest size and nest longevity (nest size: $F_{2,2} = 39.43$, $p = 0.025$; nest duration: $F_{2,2} = 76.88$, $p = 0.013$; Fig. 3-5). Neither of these dependent variables differed in relation to stocking treatment (p's ≥ 0.55). Nests defended by aggressive males were 56% larger and lasted 3 d (42%) longer than passively defended nests (Fig. 3-5). Nest size was 89% larger, and nest duration two times greater, where males were observed tending the egg masses (Passive Care and Aggressive Care combined) than for nests where no paternal care was documented ($t = 4.16\text{-}5.42$, p's ≤ 0.0002).

The proportion of nests that failed was inversely related to level of paternal care ($G_{adj.} = 27.42$, df = 2, $p < 0.0001$; Table 3-2). Nest failure was approximately 3.5 times higher in nests where males were never seen than in those where males were observed guarding nests at least one day ($G_{adj.} = 24.82$, df = 1, $p < 0.0001$; Table 3-2a). Consequently, overall number and proportion of eggs hatched were 2.3-2.4 times higher in nests where males were observed present than in No Care nests (Table 3-2a). Furthermore, these values tended to be higher for nests cared for by aggressive rather than passive males (Table 3-2a).

The relatively high proportion of nest failure in No Care and Passive Care nest categories caused the distributions of hatching success in these groups to have modes of zero. However, exclusion of failed nests normalized the data (Shapiro-Wilk test for normality: p's > 0.17), permitting the use of ANOVA. Of nests that had some egg survival, differences in the number of hatchlings among paternal care categories were only marginally significant ($F_{2,2} = 16.03$, $p = 0.06$; Table 3-2a). Hatching success, however, was similar among levels of paternal care ($F_{2,2} = 0.72$, $p = 0.58$; Table 3-2a). No difference was detected between stocking treatments in either of these models ($F_{1,1} = 1.13\text{-}1.41$, p's $= 0.45\text{-}0.48$).

Aggressive nest guarders were observed at their nests marginally (11%) more frequently than non-aggressive males ($t = 1.90$, $p = 0.061$). However, hatching success was (at least biologically) independent of the proportion of days that paternal care was observed over a nest's duration ($r^2 \leq 0.25$, df = 10-48, p's ≥ 0.001). The proportion of diseased eggs in nests was also unrelated to the proportion of days male caregivers were observed at the nests ($r^2 \leq 0.13$, df = 10-48, p's ≥ 0.18).

Size-class could be identified for 28 of the 48 (58%) parental males observed in 2004. Only one of these identifiable males was from the LS treatment. Significantly fewer Small males were aggressive, when compared to Medium and Large males ($G_{adj.}$ = 9.26, df = 2, p = 0.01; Table 3-2b). Initiation of ES nest activity was related to size-class (Kruskal-Wallis: $p < 0.002$), with Large males spawning, on average, 8 d before Small males (Dunn's test: $p < 0.002$; Table 3-2b). Hatching success was similar among size-classes of caregivers (Kruskal-Wallis: $p = 0.10$; Table 3-2b).

Discussion

Nest Activity.—Fluctuations in egg production suggested that a recovery time of 2-4 d was needed for females to replenish energy stores needed to create new batches of oocytes (see also Gale & Buynak 1982; Jensen et al. 2001). Interestingly, the pattern also indicated some synchrony in egg laying at the population-level, occurring even among pond-halves for each study year. These cyclical dynamics were possibly generated by stocking fish of identical size-classes into their respective treatment pond-halves on the same dates. Large batches laid late in the season were consistent with Gale and Buynak's (1982) report that clutch sizes in fatheads did not diminish over the spawning season.

Warmer pond water temperatures in July and August of 2003 likely explain the reduction in incubation period of LS eggs in 2003 relative to that of other treatment-years, by accelerating embryonic development (Mills 1991). Faster incubation would be expected to enhance hatching success by shortening the period that offspring remain vulnerable as eggs, when the probability of mortality is high (Bradford & Cabana 1997). However, contrary to my prediction, hatching success was independent of time of nest initiation, presumably because egg survival was controlled by factors in addition to water temperature, such as level of paternal care. LS females laid larger eggs relative to ES fish, possibly indicating a higher yolk investment into LS offspring (but see Crawford et al. 1999).

Egg survival has been reported to increase with clutch size in fathead minnow as a step function, through its concomitant association with increasing paternal care (Sargent 1988). Although the threshold above which no further gains in hatching success were evident in that laboratory study (80 eggs; Sargent 1989) was far smaller than nest sizes observed here, a correlation between hatching success and nest size was also detected in this study (with the exception of the 2004 LS treatment-year, where only 12 nests were produced).

Nest Site Selection.—The majority of nestboards that were chosen for spawning contained multiple nests, despite the availability of vacant boards in all pond-halves. This clumped spatial dispersion of territories is consistent with earlier observations that fathead minnows form nesting aggregations during the spawning season (Unger 1983; DeWitt 1993). Unoccupied nestboards in the MBRS ponds indicated that spawning substrate did not limit spawning activity of the fathead minnow populations. Indeed, total area of nestboards available per male was ~552 cm^2, far exceeding the minimum territorial space required (155 $cm^2/♂$, Flickinger 1973). Likewise, among boards having multiple nests, centers of adjacent territories were ~50 cm apart, while even closer distances have been reported (see Andrews & Flickinger 1974; Unger 1983).

One reason fathead minnow males may select nest sites that are adjacent to one another is for a greater opportunity to usurp territories from neighboring males to improve their own reproductive success (Unger 1983; Unger & Sargent 1988). Females preferentially spawn in nests that already contain eggs because eggs deposited into larger nests receive higher-quality paternal care and therefore have a greater likelihood of hatching (Sargent 1988; Jamieson 1995). Consequently, males lacking eggs are less likely to attract females, but can acquire matings by overtaking the territories of other males that possess eggs (Unger & Sargent 1988; Smith 1991). The potential for such nest evictions increases when rival males establish territories near other males and repeatedly attack residents, pressuring them to relinquish their nests (Unger 1983). Thus, intersexual selection, stemming from female oviposition choices for nest sites containing eggs (Jamieson 1995), has led to aggregated breeding territories where males battle for ownership of previously successful nests, striving to increase their own fitness.

A second, related explanation for clustered territories considers the sex pheromones that breeding males release to attract females to their nest sites (Cole & Smith 1992). Clumped breeding grounds may enhance mating success through a more concentrated release of these chemical cues. Similarly, visual courtship behaviors (see Cole & Smith 1987) from groups of mature males may better attract females than displays from solitary males.

Unlike DeWitt (1993), I observed no preference towards nest sites situated along northern shorelines. Directional preferences reported in DeWitt's (1993) study were likely incidental and due to habitat heterogeneity, i.e., higher-quality nesting grounds could have been located in the northern littoral zone in the single pond he examined. A second possibility was that northern spawning structures continued to be utilized throughout the spawning season simply as a consequence of initial spawning decisions, which led to the clustering of subsequent nest territories about the nests belonging to males that bred first. The use of several ponds in my experiment, each stripped of their natural spawning substrates, likely removed both of these underlying spatial biases in territorial establishment.

Paternal Care.—Because nests can change ownership as male fathead minnows usurp the nests of earlier breeders, resident males that I observed at nests may not have necessarily fathered all of the eggs they were guarding (see Unger & Sargent 1988 and Sargent 1989 for a more detailed description of "allopaternal care"). However, from a recruitment standpoint, the effects of nest grooming and defense on hatching success are of main interest, irrespective of whether or not the care was performed by actual fathers.

Because nest sites were scanned for guarding males only during the daily nest surveys, it is probable that some nests classified as No Care did, in fact, receive some degree of paternal care. The undetected presence of male guardians was likely responsible for the success of several No Care nests. In these nests, males may have been either temporarily absent or especially skittish and left their nests prior to nestboard inspections. As such, hatching success in nests that actually received no paternal care may be lower than what was observed in the No Care group.

The frequent occurrence of post-spawning mortality and nest abandonment by male fathead minnows added unpredictability to egg survival. Orphaned nests may have resulted from the death of the egg-guarding male. A die-off of breeding males occurred

in the ponds as the spawning season progressed, as was evident from the negligible number of adult males that were recovered at the conclusion of the experiment (see App. A). Post-spawning mortality of breeding males is common in fathead minnow (Flickinger 1973; Unger & Sargent 1988), resulting from the continual pressures associated with care and defense of nests. Alternatively, some of the nests may have been abandoned by parental males soon after spawning if they managed to acquire a more desirable territory elsewhere (Unger 1983). Nestboard monitoring was not deemed a disturbance leading to nest abandonment due to the frequent observation of guarding males returning to their nests after eggs had been counted (see also Gale & Buynak 1982).

Hatching success generally improved under paternal care. Including nests with 0% hatching success, egg survival was higher in nests where male guardians were present for at least a portion of the incubation time (Passive and Aggressive Care combined), than in nests where males were never observed (No Care). Paternal care reduced the risk of nest failure because of the protection given by caregivers to thwart predation attempts on an otherwise exposed nest, which could be entirely eaten after its discovery by schools of female minnows (pers. observ.). Surprisingly, the proportion of diseased eggs did not decrease with increasing days of observed paternal care. However, only a small proportion of eggs in the nests were infected by fungus, making predation/cannibalism a much greater threat to egg survival than disease.

Nest failure may have been higher in ES rather than LS pond-halves as a result of the greater abundance of ES breeding males, as reflected by a greater number of nests found in ES populations. Territorial conflict over control of larger nests, as observed by Unger and Sargent (1988), would have been more severe among the greater number of ES males, which may have led to more frequent nest abandonment and subsequent disappearance of the eggs by predation.

In contrast to nests lacking observed paternal care, the extended longevity of nests that were groomed by non-aggressive male fatheads ensured adequate incubation time required for at least the first clutch to hatch. Aggressive male guarders were even more successful than passive fish at maintaining breeding territories, with nests of the former lasting 3 d longer than the latter. Longer duration of nest activity allowed additional clutches to be laid over time, contributing to the tendency for aggressive males to control larger nests. Aggressive males were also observed more frequently guarding their nests than non-aggressive counterparts, presumably providing better protection and perhaps more frequent cleaning of the eggs, which subsequently decreased the likelihood of nest failure. Because of their larger nest sizes and higher egg survival, aggressive males, are expected to have higher fitness relative to passive or non-provisionary breeding males.

Differences in dates of spawning initiation and level of aggression were detected among size-classes of male caregivers. Large male minnows typically bred earlier in the season than Small males. At time of stocking, Large male minnows were 6-10 mm longer than Small males. Due to the positive relationship between body size and energy storage capacity (Cargnelli & Gross 1997; McCollum et al. 2003), these larger fish could better afford the energetic costs associated with reproduction, e.g., territorial upkeep and defense, and therefore were able to spawn earlier in the summer than smaller individuals (Baylis et al. 1993; Danylchuk & Fox 1996). Large, dominant male fathead minnows can also socially inhibit smaller males from spawning (Danylchuk & Tonn 2001), possibly by

physically preventing them from establishing nearby territories (McMillan & Smith 1974). The post-spawning mortality of these large, early-breeding males subsequently allows smaller males to commence reproductive activity later in the summer.

Differences in number and proportion of eggs that hatched were not directly evident among the three size classes, likely due to small sample sizes of males observed. Nonetheless, Medium and Large males were more likely to be aggressive than Small males, a behavioral trait that did improve egg survival. This implies an indirect link between paternal size and hatching success, with higher-quality nest care provided by larger male minnows. Furthermore, the earlier nesting activity observed in Large males, relative to that of smaller males, may have fitness implications in light of the relationships between hatch date and growth and survival of progeny. Early-hatched larvae often attain larger fall sizes than late-hatched counterparts because of the longer growing season they experience, which can translate into higher first-winter survival (e.g., Cargnelli & Gross 1996; Ludsin & DeVries 1997). Therefore, first-year recruitment may be higher for offspring spawned early in the season by larger adult fatheads than for hatchlings produced by smaller, late-spawning individuals. If adult fathead minnows survive to spawn across multiple years, their reproductive success may improve with age as they grow larger, becoming increasingly more dominant, aggressive, and earlier-spawning fish.

Conclusions

Fluctuations in recruitment documented in many fish populations may be attributed, at least in part, to interannual variability in hatching success (Bradford & Cabana 1997), highlighting the need to identify key drivers during egg development. Both paternal care and reproductive timing may co-regulate year-class dynamics in fathead minnow, but function at different life stages of the progeny. Paternal care operates more strongly at the egg stage because of its direct impacts on hatching success: lack of a male guardian often resulted in the extirpation of a nest. In contrast, the timing of nest initiation was not correlated to egg survival. Consequences of seasonal timing of reproduction on offspring may not be realized until later life stages as larvae or juveniles (see Ch. 2). Larvae that emerge relatively late in the summer have a lower growth potential before the onset of winter, which is often a size-selective barrier to first-year recruitment for many populations (Cargnelli & Gross 1996; Ludsin & DeVries 1997; Grant & Tonn 2002; Braaten & Guy 2004). Conversely, though early-season hatchlings may achieve greater first-summer size, they may also have lower fall survival than late-hatching larvae due to elevated predation mortality or sudden temperature drops in spring (Donovan et al. 1997; Garvey et al. 2002). The interaction of these stochastic factors imposes strong but interannually-variable selective pressures on hatch dates and, in turn, timing of spawning (Schultz 1993; Garvey et al. 2002). The complex variability surrounding hatching success and first-year survival makes accurate predictions of year-class strength a difficult task. Nevertheless, the egg stage may be the first in a series of critical periods in the early life history of fish and thus deserves consideration when assessing recruitment potential of new year-classes.

Table 3-1. Reproductive characteristics ($\bar{X} \pm$ SE) of Early-Stock (ES) and Late-Stock (LS) fathead minnow populations inhabiting MBRS ponds, averaged across ponds for each year. Nest failure is defined as the proportion of nests that produced zero hatchlings. Temperature measurements used for calculating seasonal averages began on the date populations were stocked and extend until the end of the nesting season.

	2003 (Ponds 3,4 & 5)		2004 (Ponds 5 & 6)	
	ES	**LS**	**ES**	**LS**
Stock date (Julian d)	24 Jun (175)	15 Jul (196)	17 Jun (169)	8 Jul (190)
Nesting season (d)	25 ± 2	19 ± 1	29 ± 2	25 ± 0
# of nests	15 ± 4	15 ± 2	25 ± 2	6 ± 0
Nest duration (d)	9.5 ± 0.7	8.1 ± 0.7	6.4 ± 0.5	11.2 ± 1.5
Nest failure	0.33 ± 0.04	0.21 ± 0.05	0.37 ± 0.07	0.08 ± 0.08
Tot. egg production (# eggs/pond-half)	34,270 ± 7,716	28,093 ± 6,599	32,304 ± 4,738	10,439 ± 3,684
Temperature (°C)	20.6 ± 0.2	22.2 ± 0.2	20.6 ± 0.2	20.2 ± 0.2
Incubation (d)	7.6 ± 0.2	6.1 ± 0.1	6.6 ± 0.2	7.4 ± 0.4
Egg diameter (mm)	-	-	1.27 ± 0.01	1.35 ± 0.01

Table 3-2. (a) Comparisons of the number of hatchlings, hatching success, and nest failure among levels of paternal care (No Care, Passive Care, Aggressive Care) observed in fathead minnow populations stocked in MBRS ponds in 2003 and 2004. The number and proportion of eggs that hatched are reported ($\bar{X} \pm$ SE) for all nests and for the subset of nests that had nonzero egg survival. The All Care category, encompassing all nests receiving any paternal care, was generated by pooling the Passive and Aggressive Care nests. (b) Comparisons of the number of hatchlings, hatching success, and aggressiveness of nesting males by size-classes in 2004. Mean and final dates of nest initiation by males of the different size-classes are also given for the ES nests of that year.

a.	All nests				Successful nests		
Level of paternal care	# hatchlings	Hatching success (%)	*n*	Nest failure (%)	# hatchlings	Hatching success (%)	*n*
No Care	442 ± 201	18.2 ± 6.3	29	69.0	1,425 ± 531	58.6 ± 12.1	9
Passive Care	803 ± 92	40.9 ± 3.7	79	24.1	1,058 ± 101	53.8 ± 3.4	60
Aggressive Care	1,457 ± 226	51.5 ± 5.4	42	11.9	1,654 ± 239	58.4 ± 5.2	37
All Care	1,030 ± 102	44.5 ± 3.1	121	19.8	1,285 ± 113	55.5 ± 2.9	97

b.	2004 nests				2004 ES nests		
Male size-class	# hatchlings	Hatching success (%)	*n*	Aggressive (# , %)	Nest initiation (Julian d ± SE)	Last nest (Julian d)	*n*
Small	864 ± 201	55.1 ± 11.2	9	1 , 11	7 Jul (189 ± 2)	17 Jul (199)	9
Medium	1,600 ± 387	73.8 ± 10.8	7	6 , 86	2 Jul (184 ± 2)	9 Jul (191)	6
Large	593 ± 195	34.6 ± 11.4	12	5 , 42	29 Jun (181 ± 1)	9 Jul (191)	12

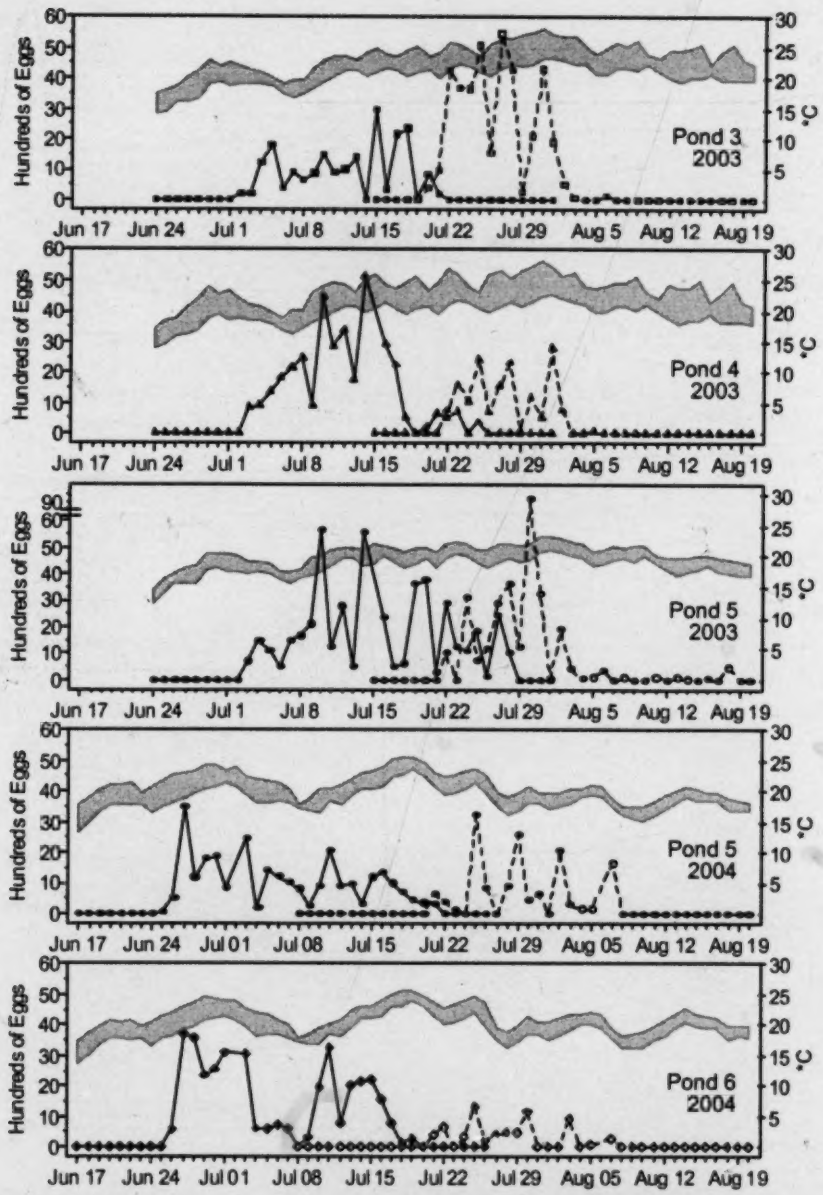

Figure 3-1. Daily egg production of Early-Stock (ES; solid shapes and lines) and Late-Stock (LS; open shapes and dashed lines) fathead minnows inhabiting MBRS ponds in 2003 and 2004. The shaded region encompasses the daily range in water temperature (°C). The initial data point for each treatment marks the stocking date: 24 June and 15 July for 2003, 17 June and 8 July for 2004 (ES and LS, respectively). Note the scale break in number of eggs produced by LS minnows in Pond 5, 2003.

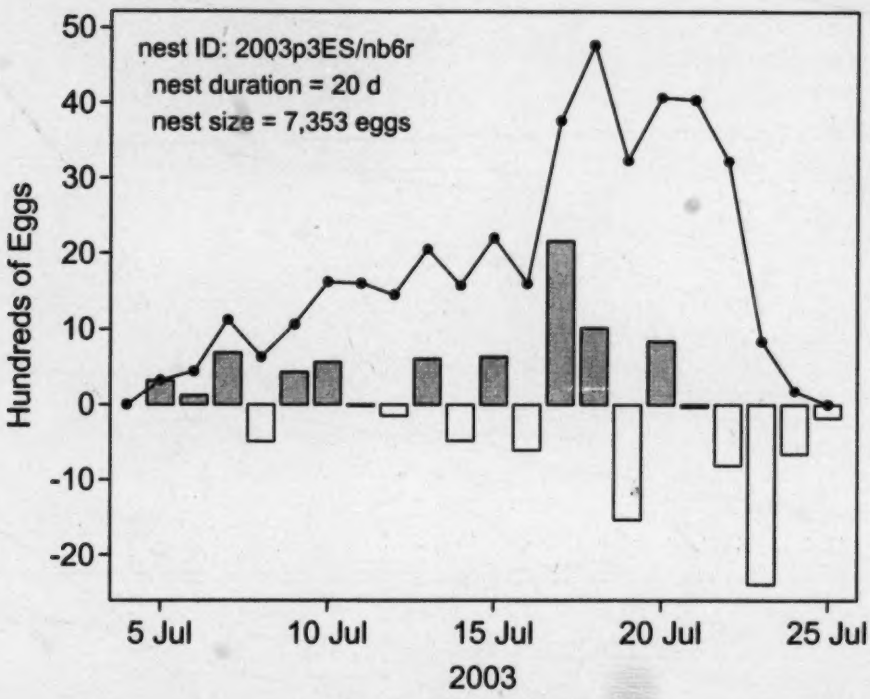

Figure 3-2. Daily change in nest size for a typical fathead minnow nest, derived from the total number of eggs (black dots) counted over consecutive days. Bars represent net gains or losses of eggs to the nest. Positive differences (shaded bars) signify the net number of eggs added to the nest. Egg losses (open bars) indicate predation, dead or diseased eggs that had been removed by the male, or hatching. Although nest longevity and total egg output varied among nests, all nests displayed similar size fluctuations.

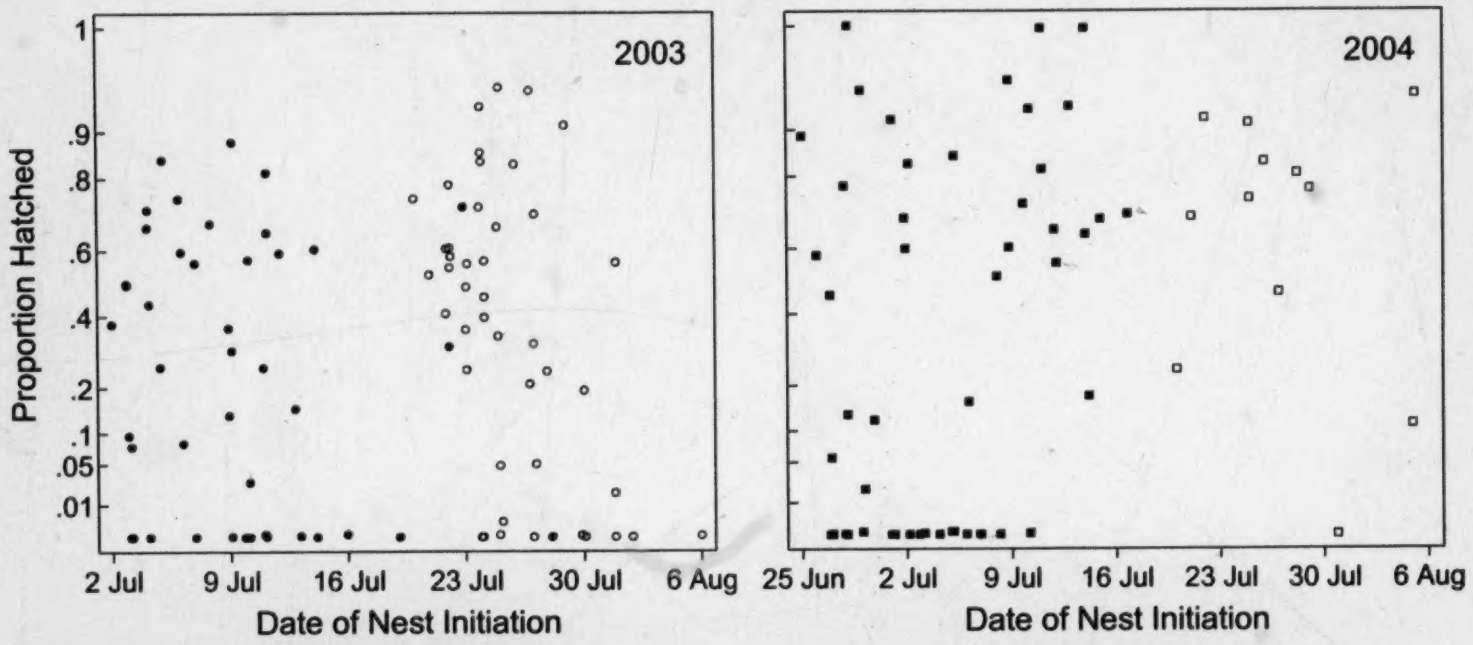

Figure 3-3. Relationships between hatching success and the date that nests first appeared during the spawning seasons of 2003 and 2004. Data are labeled as Early-Stock (ES, solid shapes) and Late-Stock (LS, open shapes) treatments. Note the differences in scale of the x-axis between years, and that the y-axes are back-transformed from their arcsine-square root values.

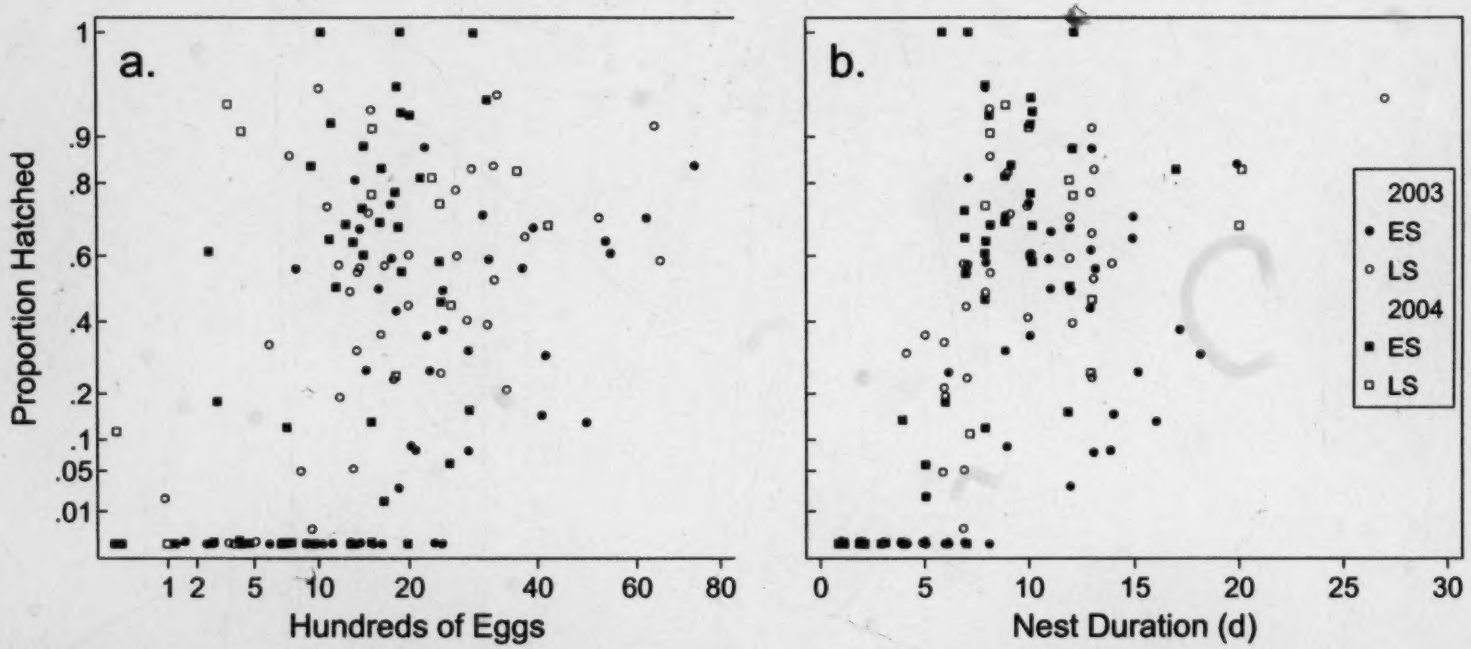

Figure 3-4. Relationships between hatching success and (a) nest size and (b) the duration of a nest. Data are labeled as Early-Stock (ES, solid shapes) and Late-Stock (LS, open shapes) treatments. Note that the y-axes are back-transformed from their arcsine-square root values, and the x-axis in (a) from its square root values.

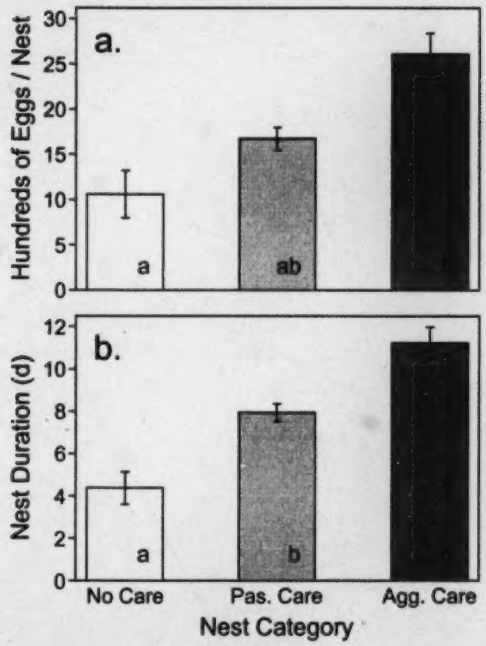

Figure 3-5. Differences in (a) nest size and (b) nest duration among nests where care was not observed (No Care; $n = 29$), nests that had males present (Passive Care; $n = 79$), and nests where males aggressively protected their nests from intruders during egg counting (Aggressive Care; $n = 42$). Data are presented as $\bar{X} \pm$ SE. Different letters denote groups that were significantly different ($p < 0.017$) as determined from Tukey-Kramer multiple comparisons. However, all pairwise comparisons were $p < 0.05$.

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Chapter 4. GENERAL DISCUSSION

The timing of reproduction and parental care can be important determinants of adult reproductive success through their effects on the survival and performance of progeny. In Chapter Two, I found that fathead minnows (*Pimephales promelas*) spawning earlier in the season are likely to have higher fitness than those that spawn later because of the greater recruitment potential of early-hatched offspring. Early-hatched minnows capitalized on a longer growing season, achieving greater length and mass relative to late-hatched counterparts. Although survival to fall of the new year-class was higher in late-hatched fish, first-winter survival was similar, and would likely be lower for late-hatched fish under conditions conducive to size-dependent mortality (see Post & Evans 1989; Cargnelli & Gross 1996; Ludsin & DeVries 1997).

Reproductive timing of adults may also affect that of subsequent generations. Because maturation is often size-dependent in fish, intra-cohort size differences can lead to variation in age-at-maturity (Fox 1994; Heino et al. 2002). Consequently, juvenile cohorts may mature at different times depending on the hatch dates of individuals. Indeed, in my experiment, early-hatched fish were more likely to mature as yearlings than late-hatched counterparts. In other fractional spawners, hatch-date induced differences in age-of-maturity has led to partial alternation of life histories: early-hatched cohorts of smallmouth bass and threespine stickleback initiated spawning earlier in life, but later in the summer, than late-hatched counterparts (Baylis et al. 1993; Saito & Nakano 1999). Although spawning dynamics of age-2 fish were not examined in this study, there is potential for a similar phenomenon to occur in fathead minnow if late-hatched progeny, having foregone reproduction as yearlings, spawn early in the summer as age-2 fish. However, the existence of true alternating life history pathways in fathead minnow would require confirmation in natural, age-structured populations.

Within years, selection for hatch dates can be directional, favoring offspring produced during a relatively short period within the longer reproductive season (e.g., Cargnelli & Gross 1996; Pine & Allen 2001). However, the optimal date for survival of offspring can shift interannually depending on current weather patterns or abundances of predator or prey (van Noordwijk et al. 1995; Donovan et al. 1997; Dzus & Clark 1998). Consequently, in the long-term, overall selection on reproductive timing may be broadly stabilizing around a mean optimum breeding date (Price et al. 1988; Anholt 1991; Schultz 1993).

Evolution of phenotypic plasticity in the timing of reproduction and protracted spawning are two solutions whereby parents can have "survival insurance" for their offspring (Garvey et al. 2002). Variation in breeding dates may also arise from non-heritable traits, e.g., energetic constraints that delay reproduction in some individuals until they achieve higher nutritional status (Price et al. 1988; Danylchuk & Fox 1994a). Similarly, younger or smaller cohorts may not mature until after the spawning season has begun (Danylchuk & Fox 1994b; Grant & Tonn 2002). Thus, it is the combination of genetic and environmentally-induced effects that influences the duration of reproductive seasons in populations.

The fundamental objective of parental care is to improve survivorship of offspring. Selection for this reproductive strategy will occur if fitness gains outweigh the costs accrued from providing the care (Gross & Sargent 1985). In Chapter Three, I have

shown that, despite suffering substantial levels of post-spawning mortality, there was a crucial benefit of paternal care: male presence at nests increased egg survival. The significance of paternal care on hatching success may, in fact, even be higher than what I recorded, since successful nests where no males were observed could have had some degree of undetected care.

Fierce males, attacking nestboard inspectors, had the lowest rates of nest failure, presumably because of higher-quality nest care. Larger breeding males were more likely to be aggressive than smaller males and also bred earlier in the season. Therefore, I expect that large, dominant males will have higher fitness than small males for two reasons, which are apart from any additional size-related differences in mating success: 1) large males have higher nest success, and 2) their offspring, by hatching early in the summer, should have higher first-year survival.

Breeding males are equipped with two major secondary sexual characteristics that help improve hatching success: a pre-dorsal pad and facial tubercles. As ceiling spawners, eggs are most often deposited on the undersides of objects. Males actively scrub their nests using their soft, mucus-secreting pad, which serves to clean and aerate the eggs (Smith & Murphy 1974). The tubercles are used in the defense of nests; males ward off nest predators by charging and head-butting them repeatedly with these horns (McMillan & Smith 1974). The combination of these nurturing and guarding elements of paternal care enabled embryos in this study to survive the vulnerable incubation period, whereas the majority of unprotected nests failed.

In addition to increasing hatching success, paternal care may be an advantageous reproductive strategy given the high winter mortality in adult fathead minnows, especially in northern populations (Danylchuk & Tonn 2003). The uncertainty of future breeding opportunities, coupled with the threat of egg predation, favors high paternal investment into nest care.

My research has demonstrated that both the timing of reproduction and parental care may be critical for recruitment success in fish, but can operate at different life stages of the progeny. Because most oviparous fishes do not care for their young after hatching, parental care is restricted to the egg stage, while the impacts of reproductive timing may not be realized until later in life. Over extended spawning seasons, parental care enhances hatching success of a series of age-0 sub-cohorts. Hatch-date related differences in growth among these sub-cohorts can shorten or prolong the duration of larval and juvenile stages, which can subsequently alter survival during these critical periods (Houde 1987). Furthermore, variation in growth rates may ultimately affect adult life histories, through its influence on age-at-maturity. Due to the far-reaching effects of reproductive timing and parental care on recruitment dynamics in fish, these parameters should be incorporated into experimental studies and population assessments.

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Appendix A. COMPARISON OF EARLY AND LATE-STOCKED EXPERIMENTAL POPULATIONS OF FATHEAD MINNOW

Introduction

The pond experiments conducted in 2003 and 2004 at Meanook Biological Research Station (MBRS) compared the performance of fathead minnow progeny spawned from Early-Stock (ES) and Late-Stock (LS) populations, which had been introduced to the ponds on different dates in the summer. Interference with natural seasonal timing of reproduction in fathead minnow was necessary to ensure a separation in hatch date between the Early-Hatch (EH) and Late-Hatch (LH) age-0 cohorts for the experiment.

Since the objectives of Chapter 2 focused on the effects of reproductive timing on recruitment, little emphasis was placed on direct comparisons between the two stocks of adult fathead minnow. However, because delays in LS reproduction were artificially imposed, such an analysis is valuable for determining whether population differences between treatments may have had any confounding effects on the experiment. Here I examine performance of the ES and LS adult fathead minnow populations, dividing the analysis into three areas: 1) demographic changes, 2) growth, and 3) reproductive investment.

Methods

Experimental Procedures.—Fathead minnows were collected from South Calling Lake 100 (SCL100) and divided into three life history categories: Male, Female, and Immature (defined as fish lacking distinguishable secondary sexual characteristics). I subdivided these categories into Small, Medium, and Large size-classes (3-mm intervals; total length, TL): ♂: S = 65-67, M = 69-71, L = 73-75; ♀: S = 58-60, M = 61-63, L = 64-66; Immature: S = 53-55, M = 56-58, L = 59-61. Fish were batch-marked by category and size-class with unique pelvic and caudal fin-clips or with acrylic paint. Male and Female fish were held in separate tanks before the experiment began to suppress spawning activity.

On 24 June 2003, ES populations of 84 fathead minnows (9-10 of each category) were introduced into one pond-half in each of three divided experimental ponds (Ponds 3, 4, and 5) at MBRS to achieve a density of approximately 1 fish/m^2, based on the average surface area of the ponds. The three LS pond-halves were stocked on 15 July, with fish having the same population structure. Herein, to make more meaningful comparisons between stocked populations, experimental "Days" correspond to time post-stocking, and therefore differ in absolute time (by 3 wk) between ES and LS treatments. Minnows found dead were replaced with similar fish from the holding tanks until Day 12 (just as spawning began), and given a unique anal fin-clip for identification. I monitored survival of stocked populations biweekly throughout the summer by setting seven funnel traps overnight, spaced evenly along shore. I measured TL and wet mass (to nearest 0.01 g) of all fish captured, and these values were used to calculate condition (K = [mass(g) / TL(cm)^3] x 100). I removed surviving stocked minnows in late August by extensive trapping.

In 2004, the experiment was repeated, with a few refinements, in Ponds 5 and 6. I collected a new sample of fathead minnow from SCL100 and fish were sorted into sex

and length categories as above. Because pond surface area was greater than in the previous year due to elevated water levels, population sizes of 95 minnows were added to the pond-halves to maintain the same fish density of the 2003 populations. Stocking of ES and LS treatments was again separated by three weeks (17 June and 8 July, respectively). I also increased the unit of sampling effort (catch per unit effort, CPUE) to nine traps set overnight. Subsamples of females were sacrificed during mid- and late-spawning periods in 2004 to determine gonadosomatic indices (GSI = ovarian mass / total mass x 100), an indicator of reproductive investment.

Statistical Analyses.—I used replicated goodness-of-fit *G*-tests to analyze relative proportions of fathead minnow categories inhabiting each pond-half (Sokal & Rohlf 1995). For each size-class and study year, mean end-of-summer length, wet mass, and condition were compared to initial values at time of stocking to calculate seasonal growth and condition of ES and LS fathead minnow. Due to small sample sizes, pond-halves were pooled within treatments to yield ~5 fish/class for the growth analysis. Average values were then paired by treatment (ES vs. LS) for the 6-7 life history categories of minnows recovered and analyzed using Wilcoxon signed-rank (*T*) tests. A two-sample *t*-test compared mid- to late-season GSI of ES Females (data were arcsine-square root transformed).

Results

Demographics.—In 2003 and 2004, average CPUE of both ES and LS minnows declined considerably over the course of the summer in all pond halves. An average of 66% of the initial populations could be trapped 1 d after stocking, dropping to 13% on Day 28 of the experiment (ES and LS combined). Intense removal trapping in late August revealed that 21% of the initial populations survived through summer.

Proportions of captured Males, Females, and Immature fish shifted as the spawning season progressed. On Day 1 of the experiment, CPUE suggested that Males were marginally more abundant of the three categories in both study years (data for 5 pond-halves: $G_T = 17.62$, df = 10, $p = 0.062$). However, relative abundance of Immature individuals began to increase between Day 14 and Day 28. By Day 42, Immature fish were disproportionately abundant, comprising 69% of the catch, (10 pond-halves: $G_T = 179.33$, df = 20, $p < 0.0001$). Mean proportions of trapped Males dropped to 5% at that time. Similarly, of all minnows harvested at the end of the experiment, only 6% were Males, compared to average proportions of 61% Immature and 33% Female fish. No Males were recovered in 6 of 10 pond-halves, and of the 10 Males removed overall, only one was of the Large size-class.

Though not a problem in 2003, immediate post-stocking mortality of fathead minnow approached 20% in LS pond-halves during 2004, and far exceeded that of ES treatments (average of 18 LS vs. 1 ES replacement fish/pond-half). However, few LS deaths were observed after the 12 d replacement period.

Growth.—Mean end-of-summer values of length, mass, and condition of stocked fatheads were similar between ES and LS fish in 2003 (fish paired by category; signed-rank tests: p's > 0.12). Lengths were again similar in 2004 ($p = 0.62$), however, LS fish were marginally heavier ($p = 0.043$) and were in better condition at the end of the summer than ES counterparts ($p = 0.018$). Overall, Immature minnows had the highest seasonal

growth, increasing 9-11 mm in length; Males and Females grew 3-8 mm over the summer (Fig. A-1). Within each life history category, summer growth was inversely related to size-class: Small fish grew 1-2 mm more, on average, than the Medium class, which tended to grow 1-2 mm more than Large individuals (Fig. A-1).

Reproductive Effort.—GSI values of Female fathead minnows decreased sharply over the course of the 2004 spawning season in both treatments. Spawning of ES fish had ended on Day 36, and mean GSI of ES Females significantly declined from 17.1% (Day 14; n = 17) to 3.7% when the ES fish were removed (Day 56; n = 15; t-test: $p < 0.0001$). Because of the already high initial mortality of the LS populations, LS Females were not sacrificed until Day 28, which was when the shorter LS spawning season had finished. Mean GSI had dropped slightly in the few LS Females captured, from 8.8% on (Day 28; n = 3) to 6.6% (Day 42; n = 2). Overall, 87% of Immature minnows began to exhibit secondary sex traits by Day 14, of which 79% were female. Though few Immature fish were sacrificed, GSI of newly mature females decreased from 9.6% (Day 14; n = 1) to 3.4% (Days 42-56; n = 3).

Discussion

CPUE trapping was an effective means of monitoring adult fathead minnow stocks; large proportions of the pond populations could be captured prior to the start of the spawning season. Progressive declines in the number of Male and Female fathead minnows captured as the summer progressed was attributed to post-spawning mortality of breeding adults, and not trap avoidance, on two lines of evidence. First, exhaustive removal harvests captured only a small percentage of the initial populations stocked. Second, several dead fish were observed during the mid-late spawning season, of which 75% (15/20) were Males (pers. observ.). Post-spawning mortality in this species results from a life history strategy that involves vigilant parental care in males and high reproductive investment in females (Flickinger 1973; Unger 1983). The die-off of breeding males coupled with depletion of oocytes in females in these small, experimental populations may explain why the ES spawning season ended in July while conditions were yet favorable for breeding, as shown by the continued spawning activity of LS fatheads into August.

Extended residence time in the holding tanks was presumed detrimental to the health of fathead minnows kept for the LS treatment in 2004, as evident by their high post-stocking mortality relative to ES populations. Although dead fish were replaced when discovered, additional unobserved mortality of LS breeding fatheads may account for the relatively few nests produced in this treatment-year (see Ch. 2). Nonetheless, growth of surviving LS fish was equal to or greater than that of ES counterparts.

By Day 14, the increased relative abundance of Immature fish and their mid-summer maturation signified that they had replaced the Males and Females as the primary spawners in both treatment populations. This demographic transition was facilitated by losses in adult stocks, releasing Immature fish from density-dependent and social-inhibitory constraints (Price et al. 1991; Danylchuk & Tonn 2001). The majority of eggs produced in the latter half of the spawning season was presumably due to the reproductive activity of these smaller/younger fathead minnows (Grant & Tonn 2002).

In conclusion, demographic changes and growth were comparable between ES and LS treatments. An overall reduction in reproductive investment in Females was observed over the summer, though I was unable to conclude whether peak GSI differed between treatments because of differences in dates sampled. From the high absolute number of eggs and progeny produced in all pond-halves (see Ch. 2), it is unlikely that the differences between ES and LS stocks would have invalidated comparisons made between EH and LH age-0 cohorts.

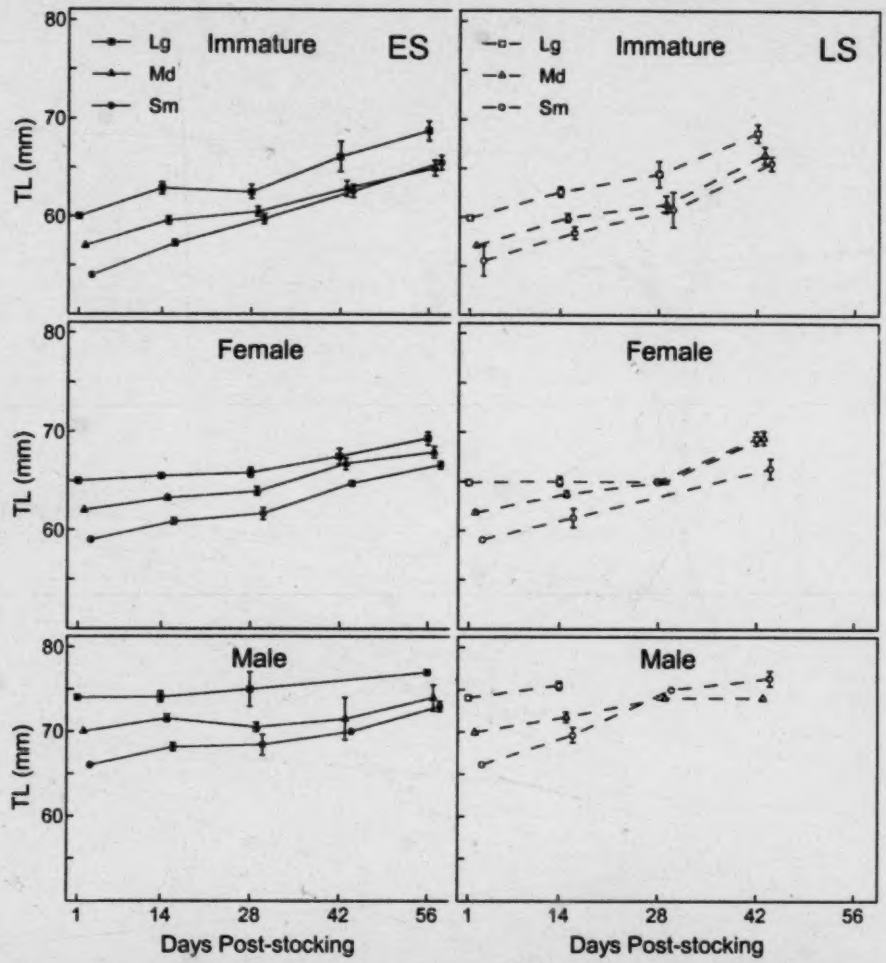

Figure A-1. Summer growth (measured in total length, TL) of Early-Stock (ES, left panels) and Late-Stock (LS, right panels) fathead minnows inhabiting MBRS ponds, grouped by life history category (Immature, Female, Male) and size-class (Large, Medium, Small). Data are presented as $\bar{X} \pm$ SE. Measurements began on Day 1, which corresponds with the stocking date of each treatment. Because of their extended residence in the ponds, ES populations were measured 14 d longer than LS fish. Data have been pooled across ponds and study years. For several sampling dates, no ES or LS large Males (and on one occasion, no small LS Females) were captured.

Appendix B. LENGTH-MASS RELATIONSHIP OF FATHEAD MINNOW

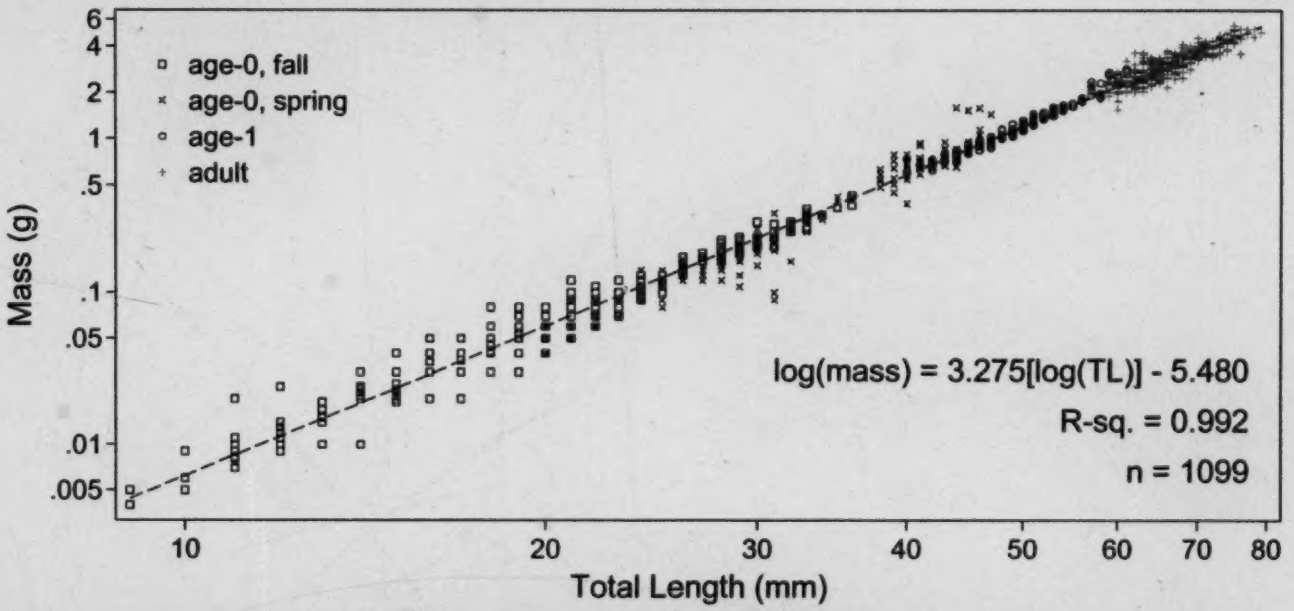

Figure B-1. Log-linear regression of total length (TL) and wet mass of fathead minnows from the MBRS pond experiments. Minnows ranged from age-0 to age-5, with TLs spanning from 9 mm to 79 mm. Adult fish (+) stocked in 2003 and 2004 from SCL100 (see Danylchuk & Tonn 2003) were selected on sampling dates that yielded the greatest catch. Because there was no increased fit in the model when either mature Males or Females were run separately, both sexes are included. Age-0 fish (2004 year-class; □) from Ponds 5 and 6 were measured in late Aug 2004. Age-0 fish (2003 year-class; x) from Pond 3 were measured after their first-winter in May 2004. Age-1 fish (○) were selected from Pond 4 in July 2004. For computational ease, the equation can be back-transformed: Wet mass (g) = 3.314×10^{-6} * TL(mm)$^{3.275}$. Note that the axes are back-transformed from their logarithmic values.

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